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(54) Title: PRION PROTEIN BINDING PROTEINS AND USES THEREOF

(57) Abstract: In general, the invention features prion protein binding proteins (PrPBPs) and diagnostic, therapeutic, and decontamination uses thereof. The invention also features fusion protein reagents for PrPBP isolation.

PRION PROTEIN BINDING PROTEINS AND USES THEREOF

Background of the Invention

This invention relates to prion protein binding proteins, nucleic acids, and uses thereof.

The prion diseases are a group of rapidly progressive, fatal, and untreatable neurodegenerative syndromes. The human prion disorders include Creutzfeldt-Jakob disease (CJD), which can be transmitted through accidental contamination of parenteral therapeutic agents (such as pituitary hormones extracted from cadavers) and transplanted tissues (such as corneas and dural grafts). To date, the Canadian Red Cross has withdrawn over 12 million dollars of blood products from the Canadian market because of potential contamination from donors who have subsequently developed CJD, or who have had family members with the disease. In addition, scrapie in sheep and goats is a common and economically important prion-related disease in North America, as is bovine spongiform encephalopathy in Great Britain. The latter also has major health and economic implications for human consumption of beef and preparation of biological products from this species.

The prion diseases are characterized pathologically by spongiform change (i.e., microcavitation of brain, usually predominant in gray matter), neuronal cell loss, astrocytic proliferation out of proportion to neuronal loss, and accumulation of an abnormal amyloidogenic protein, sometimes in discrete plaques in the brain. It is possible that neurodegeneration in prion diseases shares certain underlying mechanisms with other more common neurodegenerative syndromes such as Alzheimer's disease, amyotrophic lateral sclerosis, and Parkinson's disease.

The agents which transmit these diseases differ markedly from viruses and viroids in that no chemical or physical evidence for a nucleic acid component has been reproducibly detected in infectious materials (Prusiner, Science 216:136-144, 1982). A purification method for the scrapie agent that utilized proteinase K

digestion led to the discovery of a 27-30 kD protease-resistant protein in scrapie-affected hamster brain, termed PrP27-30 (Bolton et al., Science 218:1309-1311, 1982). PrP27-30 co-purified with scrapie agent activity and subsequently was shown to be the major (or only) macromolecule of the transmissible agent (McKinley et al., Cell 35:57-62, 1983). PrP27-30 was later determined to be a proteolytic digestion product of a 33-37 kD complete form of the scrapie agent protein (termed PrP^{Sc}), which is also capable of disease transmission.

When a partial amino acid sequence for PrP27-30 was determined and cDNAs were cloned, the gene coding for this protein was found to be host-derived (Oesch et al., Cell 40:735-746, 1985). This cellular protein has been isolated from normal brain and, unlike PrP^{Sc}, it is protease-sensitive and not associated with scrapie disease-producing activity (Bendheim et al., Ciba Found. Symp. 164-177, 1988). It is hypothesized that infectious particle-associated PrP^{Sc} is derived from a normal cellular precursor, PrP^{C} (Prusiner, Science 252:1515-1522, 1991). Recently, cell-free PrP^{Sc}-catalyzed conversion of PrP^{C} to PrP^{Sc} has been reported (Kocisko et al., Nature 370:471-473, 1994). The protease-sensitive normal cellular isoform, PrP^{C}, is an evolutionarily conserved membrane protein of unknown function. Recently, PrP^{C}, which is a glycosyl-phosphatidylinositol (GPI)-linked protein, was shown to modify T cell activation induced by concanavalin A stimulation (Cashman et al., Cell 61:185-182, 1990), suggesting an important functional role for this protein.

Transmission of prion diseases between species is limited by a "species barrier" predominantly determined by PrP amino acid sequences. Moreover, recent transgenic experiments also suggest a role for a species-specific macromolecule distinct from PrP which also participates in prion agent formation. Transgenic mice expressing high levels of human PrP^{C}, inoculated with brain extracts from humans with prion disease, were resistant to human prions. Susceptibility to human prions occurred only upon ablation of the mouse PrP gene, or expression of a human-mouse

chimeric prion gene. These findings suggest the possible existence of species-specific binding protein with higher affinity to mouse than human prion proteins (Telling et al., Cell 83:79-90, 1995). There are currently no sensitive diagnostic tests on the market and no therapeutic treatments for human prion diseases.

Summary of the Invention

In general, the invention features substantially pure prion protein binding proteins (PrPBPs). These proteins are characterized as binding in a saturable and displacable manner to a prion protein (PrP). Preferably, the binding is also of high affinity, and results in the generation of appropriate functional signals (for example, inhibition of cellular proliferation or enhanced levels of cell death). Two PrPBPs are described below; these proteins, which are isolated from murine cells, are cell surface proteins. The cloning and characterization of the genes encoding these PrPBPs is described herein. Other preferable PrPBPs may be isolated from cell or tissue samples derived from any organism, although mammals represent preferred sources and, in particular, humans and any domesticated farm animal or pet species.

Also included in the invention are purified nucleic acids which encode PrPBPs; vectors and cells containing those nucleic acids; antibodies which bind specifically to PrPBPs; and methods of producing a recombinant PrPBP involving providing a cell transformed with DNA encoding a PrPBP positioned for expression in the cell, culturing the transformed cell under conditions for expressing the DNA, and recovering the recombinant PrPBP.

Such PrPBPs are useful for the detection and treatment of prion-related diseases as well as non-prion-related diseases, e.g., degenerative syndromes such as the muscular dystrophies and disorders involving abnormal cell proliferation or abnormal cell death. These PrPBPs are also useful for the decontamination of samples known or suspected to contain prion proteins.

In another aspect, the invention also includes a fusion protein having a PrP portion and an alkaline phosphatase portion. Preferably, this protein leaves intact the

binding and functional capabilities of the PrP domain. Such PrP-AP fusion proteins provide useful affinity reagents for the labelling, detection, or identification of PrPBPs or PrP^{Sc}'s in a cell, tissue, fluid, or other biological sample.

In addition, PrP-AP fusion proteins facilitate the identification and affinity purification of PrPBP proteins in cell samples, and also facilitate the cloning of PrPBP coding sequences. In one particular method according to the invention, frog oocytes, which express minimal background surface PrPBPs are injected with *in vitro* transcribed mRNA from a cDNA library pool or clone, contacted with a PrP-AP fusion protein, and cells expressing a protein to which PrP-AP binds are identified thus allowing unambiguous identification of cDNA clones encoding PrPBP. By purifying the cDNA from those cells, the PrPBP-coding sequence may be readily recovered.

In a related aspect, the invention features methods and kits for detecting PrP^{Sc} in a biological sample. In a preferred embodiment, the method includes the steps of: (a) contacting the biological sample with a PrPBP or a fragment or analogue thereof; and (b) detecting complex formation between the PrPBP and the PrP^{Sc} of the biological sample by using a secondary PrP antibody which may be either PrP-specific (that is, an antibody which binds specifically with either PrP^{C} or PrP^{Sc}) or PrP^{Sc}-specific. If a PrP-specific antibody is used, then a threshold signal from PrP^{C} controls is established, and signals above this threshold indicate PrP^{Sc} positive samples. If a PrP^{Sc}-specific antibody is used, this control step is unnecessary as PrP^{Sc} positive samples yield positive signals while normal controls show only background signals.

In another preferred embodiment for detecting PrP^{Sc} in a biological sample, the ludes the steps of: (a) contacting the biological sample with a PrPBP; (b) destroying PrP^{C} in the biological sample (for example, by proteolytic degradation); and (c) detecting complex formation between the PrPBP and the PrP^{Sc} of the biological sample. The detection of such complex formation is taken as indicating the presence of PrP^{Sc} in the biological sample. The PrPBP used in the detection assay may be a

fusion protein (e.g., PrPBP-AP) or a PrPBP fragment or analog that binds to PrP^{Sc}. In a preferred embodiment, complex formation is detected using PrPBP (or a fragment or analog thereof) that is directly labeled. In another preferred embodiment, complex formation is detected indirectly, e.g., by using an antibody directed against PrPBP (or a fragment or analog thereof). In still another embodiment, the detection of PrP^{Sc} is determined by measuring the displacement of labeled PrP^{Sc} in a PrPBP:PrP^{Sc} complex with unlabeled PrP^{Sc} present in the biological sample. Alternatively, PrP^{Sc} may be detected in a biological sample by the steps of: (a) contacting the biological sample with a PrPBP in the presence of a limited quantity of a labeled PrP (such as PrP-AP, a conformation-dependent PrP-alkaline phosphatase fusion protein); (b) destroying PrP^{C} in the biological sample (for example, by proteolytic degradation); and (c) detecting displacement of the labeled PrP from the PrPBP complex by the unlabeled PrP^{Sc} of the biological sample. The detection of such displacement (i.e., the detection of PrP^{Sc}-PrPBP complex formation) is taken as indicating the presence of PrP^{Sc} in the biological sample.

In another related aspect, the invention features methods and kits for decontaminating PrP^{Sc} from a biological sample. In a preferred embodiment, the method involves the steps of: (a) treating the biological sample with PrPBP (or a fragment or analog thereof) or a PrPBP fusion protein for a sufficient period of time, the treatment permitting the formation of PrPBP:PrP^{Sc} complex formation; and (b) recovering the PrPBP:PrP^{Sc} complex from the biological sample. Such a decontamination method may also involve the use of perfusing a biological sample (e.g., cells or organs prepared for transplantation) with PrPBP (or a fragment or analog thereof) for the removal or inactivation of PrP^{Sc}.

In another embodiment, PrP^{Sc} bound to PrPBP (or fragment thereof) may be detected by anti-PrP antibodies.

PrPBPs which binds PrP^{Sc} can be used to quantitate PrP^{Sc} in diagnostic or other assays.

If a given PrPBP does not bind to PrP^{Sc} and instead binds only to PrP^{C}, the PrPBP can be used to remove PrP^{C} from a sample containing both PrP^{Sc} and PrP^{C}. Then an antibody which binds both PrP^{Sc} and PrP^{c} can be used to detect PrP^{Sc} remaining in the sample.

In yet another related aspect, the invention features a method of treating or preventing a prion disease in an animal (for example, a human). In a preferred embodiment, the method involves administering to the animal a therapeutically effective amount of a compound that antagonizes PrPBP:PrP^{Sc} complex formation, blocks conversion of PrP^{C} to PrP^{Sc}, or inhibits a PrPBP:PrP^{Sc} complex-mediated biological activity. In still another preferred embodiment, the method involves administering to the animal a therapeutically effective amount of a compound that antagonizes the conversion of PrP^{C} to PrP^{Sc}.

In another therapeutic approach, the invention features a method of suppressing a PrPBP:PrP^{Sc} complex-mediated biological activity in an animal. In a preferred embodiment, the method involves administering to the animal a compound (e.g., an antibody directed against PrPBP, PrPBP, or a fragment or analog thereof) that inhibits PrPBP:PrP^{Sc} complex interaction. In addition, treatment with PrPBPs may block the generation of cytotoxic signals within the body by preventing the interaction of PrP^{C} with PrP^{Sc}.

In another aspect, the invention features a method of identifying a compound for the ability to decrease a binding interaction between PrPBP (or a fragment or analog thereof) and PrP. In a preferred embodiment, the method involves the steps of: (a) mixing a compound with a PrPBP and a PrP; (b) measuring binding of the PrPBP to the PrP in the presence of the compound; and (c) identifying whether the compound decreases binding of the PrPBP to the PrP relative to a control sample.

In another therapeutic approach, the invention features a method of treating neurodegenerative syndromes by suppressing functional activation PrPBP. In a

preferred embodiment, the method involves administering to the animal a compound, e.g., small molecule antagonist or agonist, peptide, or a peptidomimetic which blocks or enhances the generation of intracellular signals from PrPBPs.

Certain diseases, e.g., neurodegenerative diseases, cancer, and immunological disorders, can be treated by administering a molecule which increases or decreases the interaction between a cadherin which is a PrPBP and PrP^C. For example, all or a PrP binding portion of a cadherin can be used to interfere with the interaction of endogenous cadherin and PrP^C. In addition compounds useful for the treatment of neurodegenerative diseases, cancer, and immunological disorders can be identified by determining whether a selected test compound increases or decreases the binding between a cadherin and PrP^c.

By "prion protein binding protein" or "PrPBP" is meant any protein which binds to a "prion protein" or "PrP" in a saturable and displacable manner. Preferably, this binding is also "high affinity" under normal physiological conditions and conformation-dependent. PrPBPs according to the invention are preferably present on the cell surface during at least a portion of their normal cellular existence, but may also be naturally present (or engineered to be present) all or part of the time in the cytoplasm, cytoplasmic organelles, or nucleus of a host cell. PrPBPs may also be present as a family of proteins in a particular cell, tissue, or organism, and PrPBPs from any organism (and, in particular, from mammals such as humans or domesticated animals, for example, sheep, cows, cats, and goats) are included in the invention.

By "high affinity" binding is meant an affinity constant (between a prion protein and PrPBP) of less than 100 μM, less than 10 μM, less than 1 μM, less than 100 nM, preferably less than 10 nM, and more preferably less than 2 nM or even 1 nM.

By "saturable" binding is meant binding (between a prion protein and PrPBP) which stops increasing after having reached a certain maximal level, indicating that there are a finite number of binding sites for one of the proteins, and that these

binding sites are specific. This is in contrast to the non-specific and continually increasing binding which is characteristic of a protein which adheres non-specifically to cell surfaces.

By "conformation dependent binding" is meant binding that occurs on a non-denatured protein that has been properly post-translationally modified, folded and transported such that its normal physiological binding characteristics remain intact.

By "competitive" binding is meant binding (between a prion protein and PrPBP) which is progressively inhibited by increasing concentrations of an unlabeled form of one of the proteins (for example, PrP).

By "prion diseases" is meant a group of rapidly progressive, fatal, and untreatable brain degenerative disorders including, without limitation, Creutzfeldt-Jakob disease (CJD), Kuru, Gerstmann-Straussler syndrome, and fatal familial insomnia in humans (Prusiner, Science 252:1515-1522, 1991), scrapie in sheep and goats, and spongiform encephalopathy in cattle, as well as recently described prion disease in other ruminants and cats.

By "treatment of prion diseases" is meant the ability to reduce, prevent, or retard the onset of any symptom associated with prion diseases, particularly those resulting in spongiform change, neuronal cell loss, astrocytic proliferation, accumulation of PrP^{Sc} protein, dementia, and death.

By "substantially pure" is meant a preparation which is at least 60% by weight (dry weight) the compound of interest. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight the compound of interest. Purity can be measured by any appropriate method, e.g., polyacrylamide gel electrophoresis or HPLC analysis.

By "purified DNA" is meant DNA that is not immediately contiguous with both of the coding sequences with which it is immediately contiguous (one on the 5' end and one on the 3' end) in the naturally occurring genome of the organism from which it is derived. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or

virus; or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., a cDNA or a genomic DNA fragment produced by PCR or restriction endonuclease treatment) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence. By "transformed cell" is meant a cell into which (or into an ancestor of which) has been introduced, by means of recombinant DNA techniques, a DNA molecule encoding (as used herein) a PrPBP.

By "positioned for expression" is meant that the DNA molecule is positioned adjacent to a DNA sequence which directs transcription and translation of the sequence (i.e., facilitates the production of, e.g., a PrPBP).

By "purified antibody" is meant antibody which is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, antibody, e.g., PrPBP-specific antibody. A purified PrPBP antibody may be obtained, for example, by affinity chromatography using recombinantly-produced PrPBP and standard techniques.

By "specifically binds" is meant an antibody which recognizes and binds PrPBP but which does not substantially recognize and bind other molecules in a sample, e.g., a biological sample, which naturally includes PrPBP.

By "alkaline phosphatase" is meant any portion of an alkaline phosphatase protein which is capable of generating a detectable signal.

As noted above, the present invention provides two very important advances for the diagnosis and treatment of prion diseases. First, the invention provides PrP fusion proteins which may be used to identify and isolate PrPBPs, and to clone the genes or cDNAs encoding these PrPBPs. In particular, one preferred PrP fusion protein makes use of an alkaline phosphatase fusion. This PrP-AP protein has numerous advantages. For example the PrP-AP protein has the advantage of minimally affecting the normal configuration of PrP, which may be necessary for binding (and, particularly, high affinity binding) to PrPBPs. Also, the PrP-AP fusion

retains the ability to trigger normal cellular PrP functions, a significant advantage over prior PrP expression constructs. Perhaps most importantly, the PrP-AP fusion protein of the invention is capable of binding specifically and with high affinity to PrPBPs. Accordingly, this fusion protein provides a useful affinity reagent to sequester or isolate PrPBPs from tissue sources. This is advantageous because it provides a means for identifying new PrPBPs and also permits rapid purification of these proteins (for example, as new protein bands on gels) for protein microsequencing as well as for cloning of the cDNA or genomic sequences. In one particular technique, a PrP-AP fusion protein may be used for the identification and cloning of new PrPBPs by screening frog oocytes microinjected with *in vitro* transcribed mRNA from a cDNA library and selecting for clones which exhibit specific binding to the fusion protein. This method of PrPBP screening is advantageous because it permits the identification of PrPBPs which are normally expressed at very low levels in cells or which may not normally be accessible to PrP-AP binding. In addition, since the PrP-AP fusion protein includes alkaline phosphatase, an easily measurable enzyme, this fusion protein provides a useful tool for the quantitation and visualization of PrPBPs under experimental or diagnostic conditions.

In another important aspect, the invention provides, for the first time, prion protein binding proteins which bind in a saturable and displacable manner to prion proteins. Such PrPBPs have numerous advantages. First, because these proteins bind specifically to PrPs, they may be labeled with detectable markers to provide an easy means to assay for the presence of PrP^{Sc} in cells, tissues, or biological fluids. Also, when labeled, these proteins may be used in drug screening protocols (for example, competitive binding assays) for the identification of other pharmacological agents which bind to PrPs. Also, because of their specific and saturable binding affinity to PrP^{Sc}, PrPBPs provide good candidates for the treatment of infected cells, tissues, or biological fluids by either removing PrP^{Sc} through secondary separation techniques or by neutralizing the recruitment activity of PrP^{Sc} in the body. PrPBPs are also

advantageous in that they may be used to treat patients afflicted with prion diseases. For example, PrPBPs, peptide fragments, or analogues thereof, when injected into the body, may be used to prevent the conversion of PrP^C to PrP^{Sc}, by binding to either of the prion proteins.

In addition to the above advantages, at least one of the PrPBPs identified herein is non-species selective and has been shown to bind to PrP from a number of different species, including human, mouse, and cow. That the PrPBP described herein binds to PrP derived from various species is likely due to the fact that the PrP epitope responsible for PrPBP binding is derived from an amino-terminal sequence which has been shown to be highly conserved.

Other features and advantages of the invention will be apparent from the detailed description, and from the claims.

Description of the Drawings

FIGURE 1A is a schematic representation of a murine PrP-AP fusion protein expression vector.

FIGURE 1B is a schematic representation of a control plasmid which expresses secreted alkaline phosphatase.

FIGURE 2 is a photographic representation of an autoradiograph illustrating that the PrP-AP fusion protein contains immunological determinants of both PrP^C and AP. ^{35}[S]-methionine labeled COS cell supernatants transfected with the CMV/SEAP vector expressing AP (lanes 1 and 2) or the APtag vector expressing PrP-AP (lanes 3 and 4) were immunoprecipitated with anti-AP monoclonal antibody (lanes 1 and 3) or PrP rabbit polyclonal antibody directed against the N-terminal 17 amino acids of the mature protein. Equivalent activities of AP were added to each immunoprecipitation reaction.

FIGURE 3 is a bar graph illustrating that PrP-AP adheres specifically to selected cell lines through the PrP^C domain. Three different mouse cell lines were cultured to confluency in 60 mm plates (approximately 100,000 cells) and were

incubated with 3 ml COS cell test supernatants at room temperature for 90 minutes, washed, lysed, and alkaline phosphatase activity determined as described below. Control: conditioned media from mock-transfected COS cells containing no detectable alkaline phosphatase activity. SEAP: supernatant from COS cells transfected with the CMV/SEAP construct, displaying no surface binding compared to control. PrP-AP: supernatants from COS cells transfected with the PrP-AP construct, displaying surface binding. SEAP and PrP-AP supernatants contained equal alkaline phosphatase activity (approximately 500 OD units per hour).

FIGURE 4 is a series of two photographic panels illustrating that PrP-AP, but not AP, recognizes a cell surface molecule by an "indirect immunofluorescence" protocol. G8 cells (a mouse muscle cell line) were mechanically dissociated without proteases, incubated with the PrP-AP or AP proteins, washed, incubated with an anti-AP monoclonal antibody, washed, incubated with goat anti-mouse immunoglobulin tagged with fluorescein isothiocyanate, washed, and examined by fluorescence microscopy. A fluorescent signal in PrP-AP-incubated cells (left panel) compared to AP-incubated cells (right panel) indicates specific cell surface binding mediated through the PrP domain of the PrP-AP fusion protein.

FIGURE 5 is a graph illustrating that PrP-AP binding is competitive and displacable through its PrP^C domain. NIH 3T3 cells in 35 mm plates were incubated as above with supernatants from COS cells transfected with the PrP-AP construct which had been metabolically labeled with ^{35}[S]-methionine. Cells were subsequently washed, lysed, and bound radioactivity was quantified by β-counting. Bound ^{35}[S]-PrP-AP is progressively competed by increasing concentrations of "cold" unlabeled PrP-AP, but not by similar concentrations of AP from CMV/SEAP-transfected COS cells.

FIGURE 6 is a series of two photographic panels illustrating a decreased cell number in G8 muscle cell line cultures exposed to PrP-AP. Sister G8 cultures in 24 well plates were incubated for three days in COS cell supernatants containing AP at

25% v/v, or COS cell supernatants containing PrP-AP 25% v/v. Cell cultures exposed to PrP-AP were consistently less dense than AP-exposed cultures, suggesting that the PrPBP binding affects cell growth and/or viability.

FIGURES 7A and 7B are the nucleic acid sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of the PrPBP of clone 6.

FIGURES 8A and 8B are graphs illustrating that an 11mer peptide from the N-terminal region of PrP can compete the binding of PrP-AP on a cell surface. FIGURE 8A indicates that a PrP 11mer peptide at 1mg/ml is sufficient to prevent 90% of PrP-AP binding. In contrast, similar amounts of another PrP peptide (Schmerling peptide, amino acids 100-130 of PrP) did not affect PrP-AP binding. FIGURE 8B shows PrP-AP binding activity as a function of the concentration of the PrP 11mer peptide used in the competition.

FIGURE 9 is a graph indicating the saturation binding curve of recombinant bovine PrP to soluble human protocadherin-43 (PC2).

FIGURE 10 is a graph showing expression of full length human PC2 in Xenopus laevis oocytes and detection by mouse PrP-AP fusion protein. Oocytes that were injected with water (neg control) and full length PC2 RNA (PC2) were probed with PrP-AP and visualized with a fluorogenic alkaline phosphatase (AP) substrate. As a positive control, elf RNA (elf) was injected and probed with its ligand mek-4-AP and visualized in the same manner.

FIGURE 11 is a graph showing the saturation binding curve of mouse PrP-AP fusion protein to soluble human PC2.

FIGURE 12 is a graph showing PC2 assay results. Two dilutions of PC2 supernatant were used to assay extracts from BSE brains and normal (NZ) brains. Absorbence values taken at 405 nm were normalized to background (PC2 - ve) and expressed as mean +/- SD, n=5.

FIGURES 13A and 13B are graphs showing PC2 distribution on lymphocytes. FIGURE 13A indicates that peripheral blood B cells (CD19 positive) coexpress PC2, unlike peripheral blood T cells (CD3 positive) in FIGURE 13B.

Detailed Description

A PrP-AP fusion protein was constructed to serve as a marker and "affinity reagent" for PrPBPs. Novel PrPBPs were detected on the surfaces of select mouse cell lines. These PrPBPs bound to the PrP portion of the PrP-AP fusion protein with high affinity, in a competitive, saturable, and conformation-dependent fashion.

Development of a PrP binding protein detection system

To develop a PrPBP detection system, a soluble recombinant fusion protein comprised of prion protein and alkaline phosphatase portions (designated PrP-AP) was constructed from a murine PrP gene in frame with secreted human placental heat-stable AP (Figure 1A). The murine PrP fragment (Locht et al., PNAS 83:6372-6, 1986; GenBank Accession #M13685) was generated by standard PCR from mouse brain cDNA and included the entire open reading frame from the initiating methionine (thus containing the PrP leader sequence) to Arg^{229} at the beginning of the GPI anchor attachment signal sequence (thus obviating the attachment of the GPI anchor). The PCR primers used to amplify this sequence are shown below:

5' AGA CAT AAG CTT GCA GCC ATC ATG GCG AAC CTT GGC 3' (forward primer) (SEQ ID NO: 3); and

5' GAG ATT GGA TCC TCT TCT CCC GTC GTA ATA G 3' (reverse primer) (SEQ ID NO: 4).

These primers were designed to contain appropriate restriction sites (forward: HindIII, reverse: BamHI) for subsequent ligation into a HindIII-BglII site of the APtag-2 expression vector (Cheng and Flanagan, Cell 79:157-168, 1994), which contains the AP gene downstream from the cassette insert site. When the PCR product is ligated into this vector, the BamHI-BglII junction generates a Gly-Ser-Ser-Gly linker between the prion portion and the alkaline phosphatase portion of the fusion protein. The APtag-2 expression vector contains an SV40 origin of replication and a CMV promoter, allowing for vector amplification and high level fusion protein production in COS cells. In order to control for possible detection of proteins binding

solely to the AP portion of the fusion protein, AP with its own leader sequence was expressed from the pCDNA1 vector (Invitrogen, San Diego, CA), also containing a CMV promotor and SV40 origin, and was designated CMV/SEAP (Figure 1B).

The PrP-AP fusion protein contains immunological determinants of both PrP^C and AP

COS cells were transfected by standard DEAE-Dextran techniques with either the PrP-AP fusion protein vector or the CMV/SEAP vector. As expected, PrP-AP-transfected COS cell supernatants (Figure 2, lanes 3 and 4) incubated with ^{35}[S]-methionine and immunoprecipitated according to standard techniques (Antibodies, A Laboratory Manual, Harlow & Lane, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988) with an AP monoclonal antibody (MIA 1801, human placenta; Medix Biotech, San Carlos, CA; lane 3) exhibited a prominent band at 97 kD, consistent with the presence of AP and the design of the PrP-AP vector. Immunoprecipitation of the same supernatants with a PrP rabbit polyclonal antibody (Bendheim et al., Nature 310:418-21, 1984; lane 4) also yielded a 97 kD band, indicating that PrP-AP-transfected COS cells secreted a 97 kD protein which was immunoreactive with both AP and PrP antibodies. Also as expected, CMV/SEAP-transfected COS cell supernatants (Figure 2, lanes 1 and 2) incubated with ^{35}[S]-methionine and immunoprecipitated with an anti-AP antibody as described above (lane 1) expressed a 67 kD protein, consistent with the molecular weight and immunoreactivity of AP. When these same COS cell supernatants were immunoprecipitated with polyclonal antibodies to PrP, no 67 kD protein was detected, indicating the absence of a prion moiety within the CMV/SEAP protein.

Both PrP-AP and AP were expressed and secreted by transfected COS cells at a concentration of about 5 μg protein per ml of supernatant, and both displayed similar supernatant AP activity (measured as described in Cheng and Flanagan, Cell 79:157-168, 994). Supernatants of untransfected and mock-transfected COS cells did not contain detectable AP activity or immunodetectable PrP^C. This data demonstrated

the successful construction and expression of the PrP-AP "affinity reagent" which was used to determine the distribution, function, and molecular identity of the PrP binding proteins.

Detection of PrPBPs on the surface of select cell lines

To determine whether the PrP-AP protein was capable of detecting PrPBPs on cells, several different cell lines including some derived from mouse, human, primate, and neuronal/neuroblastoma cell lines, were cultured with DMEM/10% fetal calf serum (FCS) to confluence in 60 mm plates (approximately 100,000 cells). These cells were washed with phosphate buffered saline (PBS) and were incubated with 3 ml of COS cell supernatants containing the PrP-AP fusion protein or the CMV/SEAP protein (i.e., lacking the PrP moiety) in DMEM/5% FCS or conditioned media from mock transfected COS cells, at room temperature for 75-90 minutes. PrP-AP-transfected cell supernatants expressed on average 500 OD units/hr of AP activity (Cheng and Flanagan, supra), indicating the presence of about 5 μg/ml of PrP-AP fusion protein. Cells were subsequently washed, lysed, heated at 65°C for 10 minutes to inactivate endogenous phosphatase, and heat stable alkaline phosphatase activity from PrP-AP determined as described above. In particular, AP activity was determined by conversion of p-nitrophenyl phosphate to a yellow product as measured by an ELISA reader (EAR 400 AT Easy Reader, SLT Instruments, Austria) at 405 nm.

Results obtained from 3 different mouse cell lines are illustrated in Figure 3. NIH 3T3 and L929 cells are derived from embryonic fibroblasts. G8 cells are myoblastoid (muscle lineage).

Also positive are human neuroblastoma lines SK-N-SH and SK-N-MC and glioma cell lines U87 and U373, human endothelial kidney (HEK) cells, primate COS cells, mouse and human peripheral lymphocytes, and mouse and human dissociated

brain cells. PrPBPs are virtually ubiquitous and do not appear to display species restricted binding. They are, however, lacking from peripheral erythrocytes and *Xenopus* oocytes.

High AP activities were detected in heat-treated G8, NIH 3T3, and L929 cell lines when incubated with the PrP-AP fusion protein derived from the supernatant of PrP-AP-transfected COS cells, (see "prpap" in Figure 3). This result indicates that PrPBPs are abundantly present on the surface of G8, NIH 3T3, and L929 cell types and are capable of binding PrP derived from the PrP-AP fusion protein with high affinity. Little or no AP activity was detected when these mouse cell lines were incubated with conditioned media from mock-transfected COS cell supernatants (i.e., in the absence of the PrP-AP fusion protein; see "control" in Figure 3). Also, little or no AP activity was detected when these cell lines were incubated with supernatants from CMV/SEAP-transfected COS cells, indicating that the detection of increased AP activity in G8, NIH 3T3, and L929 cells lines was due to the detection of specific PrPBP and not to other proteins binding only to the AP moiety of the PrP-AP fusion protein. In these experiments, SEAP and PrP-AP supernatants contained equal alkaline phosphatase activity (~500 OD units per hour). The fact that muscle cells expressed the high levels of PrPBP is consistent with their selective vulnerability in transgenic animals overexpressing PrP^C (Westaway et al., Cell 76:117-129, 1994).

To detect PrPBPs using a different technique and to determine their cellular localization, cells in suspension were labeled with the PrP-AP fusion protein using an indirect immunofluorescence assay. Cells were mechanically dissociated without proteases, triturated in Hank's Buffered Salt solution (HBSS), incubated with the PrP-AP or AP proteins for 60 minutes at room temperature, washed three times in ice-cold HBSS, and then incubated with anti-AP monoclonal antibodies (MIA 1801, human placenta, Medix Biotech, San Carlos, CA) for 30 minutes on ice. Cells were thereafter maintained at 4°C, washed again, and incubated with goat anti-mouse IgG coupled to fluorescein isothiocyanate (Jackson Immunoresearch, Philadelphia, PA), and washed and examined by fluorescence microscopy (on an Orthoplan fluorescence

microscope) or flow cytometry (Facscan; Becton Dickinson, Oakville, Ontario, Canada).

Fluorescence microscopic results for G8 cells (mouse muscle cell line) are illustrated in Figure 4. A clear labeling of the cell surface was detected in G8 cells incubated with PrP-AP protein (Figure 4, left panel), but not with the AP protein alone (Figure 4, right panel). These results indicated that specific mouse PrPBPs exist on the cell surface of G8 cells. Other cell lines, including COS cells, NIH 3T3, and L929 cells, exhibited similar results to those described for G8.

PrP-AP preparations lacking the N-terminal 10-20 amino acids (through adventitious proteolysis) did not bind to cell surfaces, implicating the PrP N-terminus in binding to its receptor. This finding was supported by peptide competition experiments (see below).

To identify the portion of the PrP which is binding to the PrPBPs on the surface of G8 and other cell lines, experiments equivalent to those described above, are performed, but in the presence of blocking domain antibodies which are specific for selected domains of the PrP protein. Particular blocking domain antibodies useful for this purpose include, for example, antibodies specific for the PrP carboxy terminus (hypothesized to determine species-specific binding by a "protein X"; Telling et al., Cell 83:79-80, 1995), antibodies specific for the middle codon region 96-176 (hypothesized to be critical in the recognition-conversion of PrP^C to PrP^{Sc} and the apoptotic properties of PrP^C peptide; Forloni et al., Nature 362:543-546), and antibodies specific for the amino-terminal octapeptide repeat region (which figures prominently in familial CJD; Prusiner, Ann. Rev. Microbiol. 48:655-686, 1994). The structural features and amino acid sequence of the binding domain is then exploited to develop other compounds which bind to PrPBP with high affinity.

PrP-AP dialyzed against EDTA and EGTA displayed no binding to cell surfaces, which was restored by addition of 1mM copper sulfate. Addition of $CuSO_4$ to PrP-AP preparations lacking the N-terminal 10-20 amino acids did not restore cell surface binding, despite retention of octapeptide domains involved in copper binding.

These data suggested that the binding reaction was not mediated by copper itself, but by some copper-induced structural change in the PrP N-terminus to facilitate binding.

PrP-AP binds equally to glycosyl-phosphatidyl inositol mutant cells, JY5 (which lack surface PrP^C), as to JY25 cells (which possess cell surface PrP^C). Thus, if PrP^C participates in homologous (self-self) binding, this cannot be the only cell surface receptor for the PrP-AP.

Characterization of PrPBP binding

To determine whether PrPBPs bound PrP in a competitive manner, binding of ^{35}[S]-labeled PrP-AP was assessed in cell lines having previously shown PrP-AP binding activity. Cells grown to confluence in 35 mm culture dishes were incubated as described previously with the supernatants of COS cells transfected with the PrP-AP fusion protein which had been metabolically labeled with ^{35}[S]-methionine. The cells were subsequently washed, lysed, and bound radioactivity quantified by β-counting.

Results for NIH 3T3 cells are illustrated in Figure 5. Binding to PrPBPs by ^{35}[S]-labeled PrP-AP was inhibited by incubation with increasing concentrations of unlabeled PrP-AP, demonstrating competitive displacement of the labeled PrP by the unlabeled PrP. Furthermore, binding to PrPBPs (by ^{35}[S]-labeled PrP-AP) was not inhibited by increasing concentrations of AP derived from the CMV/SEAP fusion protein, indicating that binding was occurring through the PrP moiety, and not the AP moiety, of the fusion protein.

To determine the binding affinity between PrPBPs and the PrP as well as the total number of binding sites present on given cell populations, Scatchard analyses were performed according to a modification of the technique of Cheng and Flanagan (Cell 79:157-168, 1994) using G8 cells. The supernatants of COS cells transfected with PrP-AP and CMV/SEAP were concentrated in an Amicon ultrafiltration cell, followed by serial dilutions in HBHA buffer (Hank's balanced salt solution with 0.5

mg/ml BSA, 0.1% NaN_3, 20 mM HEPES (pH 7.0)). After equilibrium incubation with the ligand dilution series, cells were washed extensively in HBHA, lysed, and assayed calorimetrically for bound AP activity as described above. Bound and total PrP-AP was determined for K_d affinity studies; B_{max} determination (number of binding sites per cells) was calculated from the specific activity of AP (1 pmol of PrP-AP corresponds to approximately 3 OD units under the conditions and incubation periods of the assay). The computer program LIGAND was used to plot and analyze Scatchard data. G8 cells were found to possess ~1 X 10^5 PrP-AP binding sites, with a dissociation constant (K_d) range of between 1.48 X 10^{-9} M and 25 X 10^{-9} M. This high affinity binding is consistent with a specific receptor-ligand interaction.

To determine whether the conformation of the PrP was important for high affinity binding to PrPBPs, ^{35}[S]-labeled PrP-AP was boiled at 100°C for 5 minutes to disrupt its conformation. This treatment completely ablated binding to NIH 3T3 cells, indicating that the conformation of the PrP protein is important for high affinity binding to PrPBPs.

Binding of PrP with PrPBPs transduce physiological signals such as those affecting cell proliferation and viability

To determine the physiological function of PrPBPs, the G8 muscle cell line known to possess PrPBPs was incubated with either PrP-AP or AP proteins, and proliferation of cells measured. Sister G8 cultures in 24 well plates were incubated for 3 days in COS cell supernatants containing PrP-AP or AP 25% v/v. Cells were counted in culture wells in eight high power fields after a three day incubation with the two expressed proteins. Results indicated that spontaneous proliferation of the G8 myoblast cell line was suppressed in the presence of PrP-AP (64 $\pm$ 9.8 SEM cells) compared to AP alone (283 $\pm$ 24.5 cells; p=0.002, Mann-Whitney nonparametic test).

Qualitatively, G8 cell cultures exposed to PrP-AP appeared to have more small, phase-dark, unattached cells than AP exposed cultures, suggesting that decreased proliferation may be due to increased cell death (Figure 6). The reduced

cell number observed in G8 cell cultures exposed to PrP-AP strongly suggests that binding of PrP to PrPBPs transduces a signal which inhibits cell proliferation or promotes cell death.

Characterization of PrPBPs

To verify that the PrPBPs described above were indeed proteins, sister NIH 3T3 cell cultures were incubated in calcium-free HBSS in the presence or absence of 0.25% trypsin for 30 minutes at room temperature, followed by PrP-AP incubation and calorimetric assessment of bound AP activity as described previously. This relatively mild proteolysis was sufficient to completely ablate surface PrP-AP binding to background levels ($p < 0.001$ by paired t-test). These results were consistent with the notion that PrPBPs are indeed proteins, or at least present on a protein backbone.

Library Preparation

Two pcDNA3.1 plasmid libraries were prepared from G8 cells as follows. Total RNA was extracted with TRIzol (GIBCO BRL) and polyA$^+$ mRNA was isolated using Oligotex dT (Qiagen). Synthesis of double-stranded cDNA was carried out using the Time Saver cDNA Kit (Pharmacia) with either dT or random hexamer primers. The cDNA was then blunted with T4 DNA polymerase, adapted with Bam HI oligos, and size fractioned on an S-500 column (Pharmacia). Size selected cDNAs were then ligated into pcDNAa and the libraries electroporated into high efficiency competent E. coli cells (Invitrogen) by standard techniques.

Plasmid Preparation, In Vitro Transcription, and Microinjection

Both pcDNA3.1 plasmid libraries comprised approximately 0.5×10^6 independent clones. Assuming that the mRNA abundance of the PrP ligand was "low moderate" (in the range of 0.01% of the transcripts), and that full-length cDNAs in the proper orientation would be successfully cloned in pCDNA3 at approximately 1/10 the frequency of corresponding mRNAs in the cell, we conservatively estimated

that 1-5 X 10^5 clones would have to be examined to identify a singly positive clone. The libraries were amplified in E. coli to yield pools of 1,500 to 2,000 colonies each. Individual pools were replica plated; one filter was used to grow and prepare plasmid DNA, and the other was stored. Plasmids from pools were prepared with plasmid MIDI kits (Qiagen), and mRNA was in vitro transcribed and capped from pools using mMessage mMachine (Ambion, Austin TX). *Xenopus* oocytes were prepared as previously described (Séguéla, P. et al., J. Neurosci. 16:448-455, 1996) and microinjected with 50 nL per oocyte (75-125 ng mRNA) with a nanoliter injector (World Precision Instruments).

Cloning and Characterization of PrP-BPs

We estimated that 10-20 oocytes possess the membrane area of 500,000 G8 cells, upon which PrP-AP binding can be readily detected. Oocytes were monitored for PrPBP expression at 48 hours post injection by incubating with PrP-AP supernatant at room temperature. Eggs were then washed six times in HBHA. Uniform heating to inactivate endogenous cellular phosphatases was carried out in a heating block at 65°C. Bound AP activities were detected by incubation with 0.33 mg/ml NBT and 0.17 mg/ml BCIP in AP buffer (100 mM Tris-HCl, pH 9.5, 100 mM NaCl, 5 mM $MgCl_2$) for 0.5 to 12 hours.

In all series of injections, negative controls comprised of eggs injected with vector (pcDNA3.1, Invitrogen) alone and positive controls comprised of eggs injected with mRNA prepared from a ELF-1 plasmid clone (Chang et al., 79:157-168, 1994) and detected using a MEK-4-AP fusion protein were included. After screening 33 pools (comprising approximately 660 individual Xenopus oocytes) which did not display any significant PrP binding, the 34th pool was found to be positive for PrP-AP binding. Subsequent pools were also negative for PrP binding. Pool 34 was fractionated by dilution of appropriate stock bacterial culture, into ten pools containing approximately 200 cDNA clones each. Of these, the pools demonstrating the highest level of PrP binding were chosen for further fractionation until single

positive clones were isolated. Of the 32 individual cDNA clones tested for binding activity, clone 6 exhibited the highest level of PrP-AP binding activity over background. Clone 7 exhibited moderate PrP-AP binding.

Sequencing

The cDNA inserts in clones 6 and 7 were sequenced using standard methods.

To search for homologies, the most recent updates of the EMBL and GENBANK nucleic acid databases were exploited using the BLAST network server (National Library of Medicine), and protein databases were examined using both the BLAST and the BLITZ network server (Heidelberg).

The cDNA insert in clone 6 encoded a portion of protocadherin-43 (amino acids 67-252 of protocadherin-43). The nucleic acid and deduced amino acid sequence of this portion of clone 6 is shown in Figures 7A and 7B. Protocadherin-43 is described by Sano et al. (*EMBO J.* 12:2249-2256, 1993). Protocadherin-43 sequence information is available: GENBANK L11373 (protocadherin-43).

The cDNA insert in clone 7 encoded a portion of OB-cadherin-1 (the amino terminal cadherin repeat). OB-cadherin-1 (also known as cadherin 11) is described by Okazaki et al. (*J. Biol. Chem.* 269:12092-12098, 1994). OB-cadherin-1 sequence information is available: DBJ D21253 (mouse OB-cadherin) (SEQ ID NOS: 7 and 8) and SwissProt P55287 (human OB-cadherin) (SEQ ID NO: 9).

Once a clone satisfying primary and secondary screening criteria is identified, a series of experiments are performed. For example, Northern blot analysis is carried out to reveal the size of the hybridizing mRNA species and also to determine whether there are multiple related transcripts of differing sizes. Northern blots of different cells, organs, and species, as well as blots using tissues from different developmental and disease states (particularly mouse scrapie) provide important information regarding cell specificity and regulation of the PrPBP. Discrepancies between cell surface PrP-AP binding and PrPBP expression would suggest that some cells express the PrPBP as a soluble species. In addition, using the amino acid sequences,

immunogenic sequences are identified which facilitate the production of useful antibody probes against the PrPBP. Identification of the sequence for a physiologically appropriate PrPBP also permits numerous studies which further advance the understanding of prion disease and other neurodegenerative diseases, and which enable the development of small molecule therapeutics to block interaction or inactivate this transducing molecule as a treatment of these diseases.

In the alternative, PrPBPs may be cloned by purification of the PrPBP protein using a PrP-AP affinity column and standard column purification techniques. Useful sources for PrPBPs include tissue, cell, or membrane homogenates. Following column isolation, the PrPBP is characterized biochemically, yielding information about such properties as molecular weight, pK, and glycosylation. In addition, the protein is microsequenced, facilitating database searches and cDNA cloning using degenerate oligonucleotides and standard techniques of hybridization screening or PCR amplification.

The Cadherins

The cadherins were initially recognized as calcium-dependent, homophilic cell-cell adhesion molecules, collaborating with members of other adhesion molecule families (including the integrins, immunoglobulin family adhesion molecules, and the selectins) in cell-cell recognition and adhesion phenomena in developmental formation and maintenance of tissues.

The cadherin superfamily comprises a diverse group of proteins defined by possession of extracellular "cadherin motifs" (having approximately 110 amino acids), which fold into repeated domains sharing some tertiary structural features of the immunoglobulin fold. By contrast, the cytoplasmic domains of the cadherins markedly diverge, reflecting in part the widely differing functions of these molecules. Sequence homology analysis suggests that the cadherin superfamily can be considered as at least two sub-families: the classical cadherins and the protocadherins. Others view the cadherins as falling into four functional subgroups: the classical cadherins,

the desmosomal cadherins, the protocadherins, and other cadherin-related proteins (Suzuki, *J. Cell. Biochem.* 61:531-542, 1996). A new family of brain-specific cadherins has been recently identified, designated the cadherin-related neuronal receptors (CNRs), which signal through the non-receptor tyrosine kinase fyn.

The first recognized cadherin was E-cadherin, or uvomorulin, found to mediate calcium-dependent compaction of the blastomere in early development. The original classical cadherins were all homophilic adhesion molecules with five extracellular cadherin domains. This grouping also includes M-cadherin, N-cadherin, P-cadherin, and R-cadherin. More recently discovered classical cadherins (which may constitute a subfamily) include cadherins 5-13. Several of these new atypical "classical" cadherins (including cadherin 5 and 8) do not seem to confer homophilic binding activity when transfected into L cells, although they exhibit calcium-dependent localization at points of cell-cell contact, suggesting the existence of heterophilic ligands (Suzuki, *J. Cell. Biochem.* 61:531-542, 1996). Even classical cadherins demonstrating homophilic binding may also possess heterophilic binding ligands as suggested by integrin αEß7 binding to E-cadherin (Cepek, *Nature* 372:190-193, 1994) and fibroblast growth factor receptor binding to N-cadherin.

It is likely that many heterophilic ligands of the cadherins have yet to be identified. We have demonstrated using the PrP-AP fusion protein as a detection reagent and the frog oocyte expression system that prion proteins act as novel ligands for cadherin proteins.

The adhesive and specificity-determining site of the classical cadherins is said to be contained in the most N-terminal cadherin domain, EC1 (Shapiro et al., *Nature* 374:327-337, 1995). The C-terminal end of the cytoplasmic domain of most of the classical cadherins is well conserved, reflecting their binding to catenins (Ozawa et al., 1990, Hirano et al., 1992). Exceptions to the rule are T-cadherin (cadherin-13) and a splice variant of cadherin 8. Cadherin 13 does not possess a transmembrane domain, but similar to the prion protein, is anchored to the cell surface via a glycosyl-phosphatidylinositol tail, and cadherin 8 may be secreted by cells as a

soluble isoform, completely lacking membrane anchorage of any type (Suzuki et al., 1996).

The protocadherins are a large, incompletely characterized group of proteins with homology to classical cadherins through possession of extracellular cadherin domains. However, the characterized protocadherins all appear to possess more than 5 cadherin domains, with similarity most marked to the EC3 and EC5 domains of the classical cadherins (Sano et al., *EMBO J.* 12:2249-2256, 1993). Thus, the EC1 binding motif of the classical cadherins is not shared with protocadherins; as a corollary, homophilic adhesive activity of protocadherins is not shared by all known members of this family of proteins.

Protocadherins also possess cytoplasmic domains which diverge from those of the classical cadherins, and from each other, suggesting specialized functional roles of protocadherins. It has therefore been postulated that protocadherins bind to novel and currently unknown heterophilic molecules.

The functions of the cadherins are not completely known at present. Clearly, some classical cadherins participate in cell-layer segregation and morphogenesis in development. N-cadherin plays an important role in axonal extension. Cadherins also participate in maintenance of cell-cell recognition in mature tissues, and may participate in disorders in which recognition is deficient, such as metastatic cancer. A role in tumorigenesis is suggested by the competition of the wnt proto-oncogene product for beta-catenin. Pemphigus is an autoimmune disease mediated by immune recognition of desmosomal cadherins. It is also possible that the cadherins also participate in neurodegenerative disease, muscle disease, and immunological disease. The involvement of prion proteins in binding to cadherins enables the development of agents which disrupt or enhance the actions of prion proteins on cadherin function or the action of cadherins on prion function.

Useful PrP-binding cadherins include, without limitation, E-cadherin (uvomorulin), M-cadherin, N-cadherin, P-cadherin, R-cadherin, cadherin 5, cadherin 6, cadherin 7, cadherin 8 (which may be secreted in soluble form), cadherin 9,

cadherin 10, cadherin 11 (OB-cadherin), cadherin 12, cadherin 13 (T-cadherin, which lacks an intracellular domain and is GPI-linked), PC-1 (protocadherin-42), PC-2 (protocadherin-43), protocadherin 3, protocadherin 4 (Pcdh3; GenBank Accession Nos.: AF131761 and AAD20038), protocadherin 5, protocadherin 6, protocadherin 7 (BH protocadherin; GenBank Accession Nos.: AB006755, AB006756, AB006757, and AF04364), protocadherin 8 (GenBank Accession No.: AF061573), protocadherin 9 (AA858832), OL-protocadherin (mouse; MMU88549), the cadherin-related neuronal receptors, Cnr1 (D86919), Cnr2 (D86917), Cnr3 (AB008179), Cnr4 (AB008180), Cnr5 (AB008181), Cnr6 (AB008182), Cnr7 (AB008183), and Cnr8 (AB008184), and the FAT genes from drosophila, mouse, and human (X87241). Some of these proteins are described, for example, in Sano et al., *EMBO J.* 12:2249-2256, 1993; and Suzuki, *J. Cell. Biochem.* 61:531-542, 1996.

PrPBP Binding to PrP is Calcium Dependent

Classical cadherins were first recognized by virtue of participation in homophilic cell-cell adhesion reactions which were calcium dependent. Further work has shown that calcium is not required for the adhesive interaction itself, but reacts with calcium binding sites between the cadherin folds to make the molecule more rigid and stable on the cell surface. (Shapiro et al., *Nature* 374:327-337, 1995). Even cadherin family members which do not participate in homophilic binding interactions can be induced by calcium to be localized at points of cell-cell contact (suggesting calcium-mediated binding to a heterophilic ligand).

To further investigate whether the PrPBPs originally identified on the surface of G8 and COS cells possessed the main feature of cadherin proteins--calcium-dependent binding--the following experiments were performed. PrP-AP supernatant was incubated with 2 mM EDTA for 2 hours at room temperature to chelate the calcium in the media. Concurrently, confluent COS and G8 cells were incubated in PBS containing 1mM EDTA for 5 minutes to lift cells from the bottom of the plate. Cells were then washed once in DMEM 5% FCS, and control cells

incubated in HBHA containing 1.3 mM $CaCl_2$ and 1 mM $MgCl_2$ only, while experimental cells were incubated in PBS containing 1mM EDTA at room temperature for 30 minutes to remove calcium in the media. The first group of negative control cells were pelleted and resuspended in DMEM 5% (devoid of PrP-AP). The second group of positive control cells (calcium present in medium) and a third group of experimental cells (treated with EDTA) were resuspended in EDTA-treated PrP-AP for 1.5 hours at room temperature, followed by three washes in cold HBHA. The cells were then lysed as previously described and their supernatants examined for PrP-AP activity.

Binding of PrP-AP to both COS and G8 cells was significantly reduced in the presence of the calcium chelator EDTA ($p<0.0001$ for both COS a nd G8 cells, N=6) indicating the requirement for the presence of divalent cations such as Ca^{2+} for optimum binding.

PrPBP Binding to PrP Displays Calcium Dependent Trypsin Resistance

Calcium binding protects some cadherins from trypsin digestion (Takeichi, *J. Cell. Biol.* 75:464:474, 1977), probably by inducing a conformational change which masks protease-sensitive sites. To investigate whether the PrPBP originally identified on the surface of G8 and COS cells possessed a main feature of cadherin proteins--resistance to proteolysis in the presence of calcium--the following experiments were performed. Confluent COS and G8 cells were incubated in PBS containing 0.25% trypsin and 10 mM $CaCl_2$ for 20 minutes at 37°C or PBS containing 0.25% trypsin and 1 mM EDTA and then washed three times in DMEM 5% FCS. Approximately 1 x 10^6 cells were distributed per Eppendorf tube, and these were washed twice with HBHA media. The cells were then incubated with PrP-AP supernatant for 1.5 hours at room temperature, followed by three washes in cold HBHA. The cells were then lysed on ice in 50 mM Tris, 150 mM NaCl, 1% Triton-X100, 0.02% NaN_3 pH 8.0. The lysate was microcentrifuged, and the supernatant was uniformly heated on a heating block to 65°C for 12 minutes to inactivate endogenous cellular phosphatases.

Bound AP activity was detected by incubation with NBT and BCIP in AP buffer (100 mM Tris-HCl, pH 9.5,10 0 mM NaCl, 5 mM $MgCl_2$ for 0.5 to 12 hours).

In the presence of Ca^{2+} ions PrP-AP binding to COS and G8 cells was maintained, despite treatment of the cells with trypsin prior to their incubation with PrP-AP. In the absence of Ca^{2+}, trypsinization produced a significant reduction of PrP-AP binding ($p<0.001$ for COS and $p<0.05$ for G8 cells, N=4). This data indicates that the PrPBP species on the surface of the COS and G8 cells is protected from trypsin proteolysis in the presence of Ca^{2+}, a known characteristic of cadherin molecules (Takahashi et al., *Oncogene* 8:2925-2929, 1993). This data therefore supports the notion that PrPs bind to cadherin family members.

Isolation and cloning of a human PrPBP

Human PrPBP may be isolated as described above for the murine homolog. In particular, a recombinant human PrP-AP fusion gene is constructed essentially as described above, using the human PrP cDNA described by Kretzschmar et al. (DNA 5:315-324, 1986) in place of the murine PrP gene. The human PrP fragment is generated by standard PCR amplification using the following primers:
5' AGA CAT AAG CTT GCA GCC ATC ATG GCG AAC CTT GGC 3' (forward primer) (SEQ ID NO: 5); and
5' GAG ATT GGA TCC TCT CTG GTA ATA GGC CTG 3' (reverse primer) (SEQ ID NO: 6).
This fragment is then cloned in-frame into the APtag-2 expression vector (Cheng and Flanagan, supra). Using the fusion protein product expressed from this vector, human PrPBPs are isolated by any of the affinity techniques described above (for example, by precipitation from human PrPBP-producing cells). Verification that the protein isolated is, in fact, a human PrPBP may also be accomplished as described above.

Once isolated, the human PrPBP protein may be microsequenced, and the amino acid sequence used to design oligonucleotides for hybridization screening or PCR primers for amplification of the human PrPBP coding sequence from any appropriate human cDNA or genomic DNA library.

Alternatively, the human PrP-AP fusion protein is used to isolate a human PrPBP-expressing cDNA clone using the pcDNA3.1 cloning system and the techniques described above. Or, preferably, in yet another alternative technique, a reduced stringency screening of a human brain cDNA library is performed using the mouse PrPBP coding sequence (described above) as a probe. Again, mouse PrPBP sequences may be used as hybridization probes, or PCR primers may be designed based upon regions of the mouse PrPBP sequence likely to be conserved across different species and used for amplification of the human sequence. Finally, if desired, homologs of mouse PrPBPs may be tested for activity in PrP-AP assays, as described above.

PrPBP Protein Expression

In general, PrPBPs according to the invention may be produced by transformation of a suitable host cell with all or part of a PrPBP-encoding cDNA fragment in a suitable expression vehicle.

Those skilled in the field of molecular biology will understand that any of a wide variety of expression systems may be used to provide the recombinant protein. The precise host cell used is not critical to the invention. The PrPBP may be produced in a prokaryotic host (e.g., E.coli) or in a eukaryotic host (e.g., Saccharomyces cerevisiae, insect cells, e.g., Sf21 cells, or mammalian cells, e.g., NIH 3T3, HeLa, or preferably COS cells). Such cells are available from a wide range of sources (e.g., the American Type Culture Collection, Rockland, MD; also, see, e.g., Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, New York, 1994). The method of transformation or transfection and the choice of expression vehicle will depend on the host system selected. Transformation and

transfection methods are described, e.g., in Ausubel et al. (supra); expression vehicles may be chosen from those provided, e.g., in Cloning Vectors: A Laboratory Manual (P.H. Pouwels et al., 1985, Supp. 1987). Alternatively, a PrPBP may be produced by a stably-transfected mammalian cell line. A number of vectors suitable for stable transfection of mammalian cells are available to the public, e.g., see Pouwels et al. (supra); methods for constructing such cell lines are also publicly available, e.g., in Ausubel et al. (supra). In one example, cDNA encoding the PrPBP is cloned into an expression vector which includes the dihydrofolate reductase (DHFR) gene. Integration of the plasmid and, therefore, the PrPBP-encoding gene into the host cell chromosome is selected for by inclusion of 0.01-300 μM methotrexate in the cell culture medium (as described in Ausubel et al., supra). This dominant selection can be accomplished in most cell types. Recombinant protein expression can be increased by DHFR-mediated amplification of the transfected gene. Methods for selecting cell lines bearing gene amplifications are described in Ausubel et al. (supra); such methods generally involve extended culture in medium containing gradually increasing levels of methotrexate. DHFR-containing expression vectors commonly used for this purpose include pCVSEII-DHFR and pAdD26SV(A) (described in Ausubel et al., supra). Any of the host cells described above or, preferably, a DHFR-deficient CHO cell line (e.g., CHO DHFR$^-$ cells, ATCC Accession No. CRL 9096) are among the host cells preferred for DHFR selection of a stably-transfected cell line or DHFR-mediated gene amplification.

Once the recombinant PrPBP is expressed, it is isolated, e.g., using affinity or ion exchange chromatography. In one example, an anti-PrPBP antibody (e.g., produced as described herein) may be attached to a column and used to isolate the PrPBP. Lysis and fractionation of PrPBP-harboring cells prior to affinity chromatography may be performed by standard methods (see, e.g., Ausubel et al., supra).

Once isolated, the recombinant protein can, if desired, be further purified, e.g., by high performance liquid chromatography (see, e.g., Fisher, Laboratory Techniques

In Biochemistry And Molecular Biology, eds., Work and Burdon, Elsevier, 1980).

Polypeptides of the invention, particularly short PrPBP fragments, can also be produced by chemical synthesis (e.g., by the methods described in Solid Phase Peptide Synthesis, 2nd ed., 1984 The Pierce Chemical Co., Rockford, IL).

These general techniques of polypeptide expression and purification can also be used to produce and isolate useful PrPBP fragments or analogs (described herein).

Particularly useful fragments of the invention include extracellular PrPBP domains, for example, protocadherin-43 (PC2) amino acids 29-244, as well as the smaller fragments EC1 (amino acids 29-135) and EC2 (amino acids 136-244).

Anti-PrPBP Antibodies

To generate PrPBP-specific antibodies, a PrPBP coding sequence may be expressed as a C-terminal fusion with glutathione S-transferase (GST) (Smith et al., Gene 67:31-40, 1988). The fusion protein is purified on glutathione-Sepharose beads, eluted with glutathione, cleaved with thrombin (at the engineered cleavage site), and purified to the degree necessary for immunization of rabbits. Primary immunizations are carried out with Freund's complete adjuvant and subsequent immunizations with Freund's incomplete adjuvant. Antibody titres are monitored by Western blot and immunoprecipitation analyses using the thrombin-cleaved PrPBP protein fragment of the GST-PrPBP fusion protein. Immune sera are affinity purified using CNBr-Sepharose-coupled PrPBP protein. Antiserum specificity is determined using a panel of unrelated GST proteins.

As an alternate or adjunct immunogen to GST fusion proteins, peptides corresponding to relatively unique immunogenic regions of PrPBP may be generated and coupled to keyhole limpet hemocyanin (KLH) through an introduced C-terminal lysine. Antiserum to each of these peptides is similarly affinity purified on peptides conjugated to BSA, and specificity tested in ELISA and Western blots using peptide conjugates, and by Western blot and immunoprecipitation using PrPBP expressed as a GST fusion protein.

Alternatively, monoclonal antibodies may be prepared using the PrPBPs described above and standard hybridoma technology (see, e.g., Kohler et al., Nature 256:495, 1975; Kohler et al., Eur. J. Immunol. 6:511, 1976; Kohler et al., Eur. J. Immunol. 6:292, 1976; Hammerling et al., In Monoclonal Antibodies and T Cell Hybridomas, Elsevier, NY, 1981; Ausubel et al., supra). Once produced, monoclonal antibodies are also tested for specific PrPBP recognition by Western blot or immunoprecipitation analysis (by the methods described in Ausubel et al., supra). Antibodies which specifically recognize PrPBP are considered to be useful in the invention; such antibodies may be used, e.g., in an immunoassay to monitor the level of PrPBP produced by a mammal (for example, to determine the amount or subcellular location of PrPBP). Alternatively monoclonal antibodies may be prepared using the PrPBP described above and a phage display library (Vaughan et al., Nature Biotech 14:309-314, 1996).

Preferably, antibodies of the invention are produced using fragments of the PrPBP which lie outside generally conserved regions and appear likely to be antigenic, by criteria such as high frequency of charged residues. In one specific example, such fragments are generated by standard techniques of PCR and cloned into the pGEX expression vector (Ausubel et al., supra). Fusion proteins are expressed in E. coli and purified using a glutathione agarose affinity matrix as described in Ausubel et al. (supra). To attempt to minimize the potential problems of low affinity or specificity of antisera, two or three such fusions are generated for each protein, and each fusion is injected into at least two rabbits. Antisera are raised by injections in a series, preferably including at least three booster injections.

Cross-Species PrPBP Binding

As discussed above, at least one of the exemplary PrPBPs identified herein, that is, PC2 (also referred to as protocadherin-43) is non-species selective and binds

to PrP from a number of animals including human, mouse, and cow. Also, as discussed below, this is likely due to the fact that this PrPBP binds to a highly conserved amino-terminal PrP peptide.

In our experiments, peptide competition studies indicated that this highly conserved 11 amino acid peptide derived from the distal amino terminus of the prion protein was capable of significantly inhibiting PrP-AP binding to the surface of COS and G8 cells, which were shown to be rich in PrPBPs. In these studies, G8 cells were plated in 6-well plates at approximately 100,000 cells per well. 200μl of either the PrP 11mer peptide (KKRPKPGGWNT) or the control Schmerling peptide (PrP amino acids 100-130) were added from a stock of 10mg/ml to 1.9ml of PrP-AP diluted 1:10 in Grace's media. This mixture, which contained 1mg/ml peptide in 2ml final volume of 1:10 diluted mouse PrP-AP, was added to the G8 cell-containing wells and allowed to incubate for 90 minutes at room temperature. The cells were then washed 6X with 2ml HBHA and lysed with 400ml of lysis buffer (100mM NaCl, 10mM EDTA, 0.5% Nonidet P-40, 0.5% sodium deoxycholate in Tris-HCl, pH 7.4) for 15 minutes at room temperature. The mixture was then incubated on ice for 15 minutes and then centrifuged to remove cellular debris. The supernatants were transferred to fresh tubes and incubated at 65°C for 12 minutes to inactivate endogenous AP activity. PrP-AP was visualized by incubation with BCPIP substrate (Kirkegaard), which yields a colored reaction product that is quantitated at 620nm. The competition binding of PrP-AP to COS cells was done in a similar manner, with the following exceptions: 5000 cells were plated per well into 96-well plates, and a titration of the PrP 11mer peptide was used, with concentrations starting at 1mg/ml and decreasing by 3-fold serial dilutions.

The PrP 11mer competed 90% of the binding of PrP-AP to G8 cells, whereas the control Schmerling peptide did not show any effect (Figure 8A). Furthermore, the competition of PrP-AP binding by the PrP 11mer was dose dependent, as

progressively less PrP peptide used resulted in progressively less competition (Figure 8B). These results strongly indicated that this 11 amino acid stretch of PrP was involved in binding of PrP to its cell surface receptor.

Binding of the Human PrPBP, PC2, to Bovine PrP

The soluble human PrPBP, soluble PC2, used in all experiments was made by transiently transfecting COS cells with a plasmid which contained the PC2 gene lacking its transmembrane domain. This plasmid was called Hu-PC43 3'trunc/PC-1 neo. It contained all 6 extracellular domains of PC2 and a neomycin resistance gene as a selectable marker. Culture supernatants were harvested after 5 days of culture and were tested for the presence of PC2 by ELISA. Positive supernatants were then aliquoted and frozen at -80°C. Mouse PrP-AP was produced after baculovirus infection of SF9 cells and used as a supernatant.

To generate saturation binding curves, 50μl of PC2 supernatant, diluted 1/3 in Dulbecco's PBS containing 1mM Ca^{2+} and Mg^{2+}, were laid down in a 96-well Immunolon 1B plate (Dynex) and incubated overnight at 4°C. As a control, a 1/3 dilution of COS supernatants containing an irrelevant protein (mek4-AP) were also laid down in the same manner. After an overnight incubation, non-specific binding sites were blocked with 100μl per well of Tris buffer (50mM Tris HCl pH 8.0, 150mM NaCl, 1mM Ca^{2+}) containing 5% powdered milk for 2 hours at room temperature (RT). The wells were then washed 5 times with 200μl per well of Tris buffer containing 0.05% Tween, and 50μl recombinant bovine PrP (rbPrP, Prionics) made up in Tris buffer containing protease inhibitors (Sigma P8340) were added per well. The concentrations of rbPrP used began at 2000ng/ml, then 1500ng/ml, and proceeded in two-fold dilutions until it reached 1.6ng/ml. No rbPrP was added to the last well. Each concentration was done in triplicate, and the incubations were carried out for 2 hours at room temperature. The wells were then washed 10 times with 200μl per well of Tris buffer containing 0.05% Tween and were incubated with 50μl of 6H4 anti-PrP monoclonal antibody (Prionics) diluted 1/5000 in Tris buffer

containing the protease inhibitors for 1 hour at room temperature. After incubation, the wells were washed 10 times with 200μl per well of Tris buffer containing 0.05% Tween and were incubated with a goat anti-mouse Ig-HRP conjugate (Jackson, 1/10,000 dilution) in Tris buffer containing the protease inhibitors for 40 minutes at room temperature. Following another series of 10 washes with 200μl per well of Tris buffer containing 0.05% Tween, 100μl of ABTS enzyme substrate (Boehringer) was added to the wells for 30-60 minutes at room temperature, and the optical density of the solution was measured at 405nm. Specific binding was determined by comparing the results from the PC2-containing wells to the results from the mek4-AP (irrelevant protein) wells. Other controls done in triplicate per experiment included the direct measurement of PC2 captured per well using an anti-PC2 monoclonal antibody diluted 1/10,000 in Tris buffer containing the protease inhibitors for 1 hour at room temperature, followed by a wash and incubation with the goat anti-mouse Ig-HRP conjugate as described above.

Figure 9 indicates that the binding of rbPrP to soluble human PC2 was saturable. Saturable binding is one of the hallmarks of a specific ligand-receptor interaction. The saturation binding curve was repeated 4 times. The data indicated that human soluble PC2 bound bovine recombinant PrP in a statistically significant and saturable manner. Furthermore, as saturation binding of rbPrP was reached at approximately 200ng/ml, the affinity of soluble human PC2 to rbPrP could be estimated from this data to be approximately 4 nM. A representative saturation binding curve is shown in Figure 9.

Expression of the Human PrPBP, PC2, in Xenopus laevis Oocytes and Detection by Mouse PrP-AP Fusion Protein

A plasmid containing the full length human PC2 was linearized by restriction enzyme digestion, and PC2 RNA was transcribed *in vitro* using the Ambion message machine kit. Fifteen ng of PC2 RNA were individually microinjected into freshly prepared Xenopus oocytes. Eight pools of 20 eggs each were injected with PC2 RNA,

2 pools of 20 eggs were injected with the positive control, elf RNA, made in the same manner, and 2 pools of 20 eggs were injected with buffer alone, as negative controls. The eggs were incubated at 18°C in Barth's media for 5 days to allow protein expression. Following this period, the egg pools were harvested, distributed into a 24-well plate, and incubated with mouse PrP-AP fusion protein diluted 1/10 in Grace's media for 4 hours at room temperature with gentle shaking. The positive controls were incubated with undiluted mek4-AP supernatant, whereas the mock-injected controls were incubated with PrP-AP. All the oocytes were then transferred to 1.5ml tubes and washed 6 times with cold HBHA buffer, lysed with 50mM Tris pH 8.0, 150mM NaCl, 0.02% azide, 1% Triton X-100 for 15 minutes on ice, and the supernatants were collected by centrifugation. The supernatants were then incubated with alkaline phosphate substrates, either BCPIP (Kirkegaard) which yields a colored product, or fluorescein diphosphate (F-dP, Molecular Probes) which yields a fluorescent product. The optical density of each sample was measured at 620nm, or the fluorescence of each sample was measured at 535nm.

Mouse PrP-AP fusion protein was found to bind human PC2 introduced into Xenopus oocytes and expressed on the cell surface. In the controlled system, where a known pair of ligand-receptor molecules were used, elf RNA-microinjected oocytes demonstrated statistically significant binding to mek4-AP fusion protein as compared to oocytes injected with buffer alone (p-value = 0.026, Figure 10). Likewise, there was statistically significant binding of mouse PrP-AP to oocytes microinjected with PC2 RNA (p-value 0.001, Figure 10).

Binding of the Human PrPBP, PC2, to Mouse PrP

To carry out these experiments, mouse PrP-AP fusion protein and soluble human PC2 were produced as described above, from a baculovirus expression system and transient COS cell infection, respectively. The saturation binding curve

experiment was carried out as described for bovine PrP, except that brPrP was replaced with mouse PrP-AP supernatant, which was used undiluted and in serial 3-fold dilutions ending in a final dilution of 1/300.

Mouse PrP-AP was shown to bind full length human PC2 that was expressed on the cell surface of Xenopus oocytes. This experiment demonstrated that mouse PrP-AP was able to bind to the engineered soluble version of human PC2 lacking a transmembrane domain. Indeed, mouse PrP-AP displayed a saturation binding to soluble human PC2 that was superior to the BSA control, and with statistical significance (p-values for all data points < 0.001, Figure 11). Saturation binding was reached at 1/3 dilution of the mouse PrP-AP supernatant, estimated at that dilution to contain approximately 3μg/ml mouse PrP-AP. Half maximal binding was reached at 1/10 dilution of the supernatant, which results in an estimated affinity of PrP-AP for PC2 in the low nanomolar range. Note that both binding partners were used in supernatant form, their precise respective concentrations were not known, and therefore it is likely that one or the other component was present in limiting quantities.

Detection of PrP in Bovine Spongiform Encephalopathy-Infected Brain Extracts

Brain homogenates from cows diagnosed with bovine spongiform encephalopathy (BSE) and control cows from New Zealand (i.e., bovine spongiform encephalopathy-free) were prepared as a 10% (w/v) solution. Bovine brain was disrupted in a Dounce homogeniser (with a Teflon pestle) in 2 volumes of cold lysis buffer (100mM NaCl, 10mM EDTA, 0.5% Nonidet P-40, 0.5% sodium deoxycholate in Tris-HCl, pH 7.4). The sample was incubated on ice for 20 minutes before applying 15 additional strokes in the homogeniser. Soluble debris was removed by centrifugation at 3000 r.p.m. for 15 min. at 4°C. The supernatant was aliquoted and stored at -70°C. The protein content of the supernatant was quantitated, a procedure which consistently yielded a homogenate at a concentration of ~10 mg/ml.

To assay for binding, the wells of a PolySorp ELISA plate (Nunc) were coated overnight at 4°C with a PC2-containing culture supernatant (in 0.1 M bicarbonate buffer, pH 9.6) prepared as described above. The wells were washed four times with PBS containing 0.05% Tween 20, and blocked by filling the wells with 5% nonfat milk in PBS and incubating the plate at room temperature for 2 hours. The plates were washed, and the brain homogenate (diluted in PBS) was added to designated wells and incubated at room temperature for 1.5 hours. Wells were washed four times with PBST. 6H4 (70 ng) was added to appropriate wells and incubated at room temperature for 1 hour, followed by a further 0.5 hour incubation with 100μl of an anti-Rabbit IgG/horseradish peroxidase conjugate (1:5000) in PBST containing 1% nonfat milk. Wells were washed four times with PBST. After the addition of ABTS (Boehringer Mannheim) to the wells, the signal was monitored at 405 nm.

This assay indicated an interaction between components in the brain extracts and the sPC2-containing supernatant coated on PolySorp ELISA plates (Figure 12). A dose-responsive effect was observed for both the sPC2 supernatant and for the brain homogenates. In addition, an increased level of binding was observed with extracts from BSE brain as compared to extracts from normal (NZ) brain, suggesting that PC2 may bind more avidly to PrP^{Sc}. In these experiments the signal on brain homogenates bound to pre-blocked wells did not exceed background, suggesting minimal non-specific protein-protein interactions.

PC2 Lymphocyte Distribution

To better understand the role of PC2, its distribution in the lymphoid system was examined. Peripheral blood leukocytes (PBL) were isolated from whole blood by ficol centrifugation. The PBL were washed twice in Dulbecco's PBS supplemented with 2.5% FBS. Approximately $1x10^6$ cells were distributed per well of a round bottom 96-well plate and were pelleted by centrifugation. The cells were resuspended in an anti-PC2 monoclonal antibody solution containing 10μg/ml of the antibody in Dulbecco's PBS, 2.5% FBS. A negative control population was incubated with an

isotype matched irrelevant antibody. The cells were incubated with the antibodies for 15 minutes on ice and then washed twice with 200μl of Dulbecco's PBS, 2.5% FBS. The cells were then incubated with the secondary antibody, a goat anti-mouse Ig FITC conjugate (Jackson), for 15 minutes on ice and washed in the same manner as before, followed by an incubation with normal mouse serum (Sigma), diluted 1/20, to block free antibody binding sites on the secondary FITC-conjugated antibody. The wells that were counterstained for B cells were then incubated with PE-conjugated anti-human CD19 (Becton Dickinson), while those that were counterstained for T cells were incubated with PE-conjugated anti-human CD3 (Becton Dickinson). The cells were analyzed on a Becton Dickinson FACScan. Fluorescence emissions from 10,000 live cells were collected.

The data, which is shown in Figures 13A and 13B, clearly indicated that PC2 was co-expressed exclusively with $CD19^+$ cells (B cells), but was not expressed on cells bearing cell surface CD3 (T cells). This finding is significant in light of recent results demonstrating that B cells are responsible for trafficking PrP^{Sc} to the brain following peripheral inoculation (Klein et al., Nature 390:687, 1997). The presence of both PC2 and PrP^{Sc} in these cells at early stages of prion infection lends credence to the notion that PC2 may bind PrP^{Sc} with high affinity and might be involved in the conversion of normal cellular PrP^{C} to PrP^{Sc}, and its trafficking from the peripheral lymphoid tissue to the brain.

Prion Disease Detection Assays

PrPBPs find diagnostic use generally in the detection or monitoring of prion diseases or conditions. For example, PrPBPs may be used to monitor the presence or absence of PrP^{Sc} in a biological sample (e.g., a tissue biopsy or fluid) using standard detection assays. Such detection assays include, but are not limited to, those that rely on detectably-labeled components, for example, a labeled PrPBP, a labeled PrPBP fusion protein, a labeled PrP^{C}, or a labeled PrP^{Sc}. Assays according to the invention may involve direct detection of a PrP^{Sc} or may involve indirect detection (for

example, by binding PrPBP to a PrP and then detecting the complex using a second labeled antibody directed against PrPBP, PrP, or PrP^{Sc}). PrPBPs (for example, mouse or human PrPBPs) for use in these detection assays may be produced recombinantly in any cell, but COS cells are a preferred host cell (e.g., according to the methods of Cullen, Meth. Enzymol. 152:684-704, 1988). Alternatively, the PrPBP may be isolated by affinity purification using a PrP-AP protein and standard techniques. In one particular preferred embodiment, soluble PrPBP binding domains are expressed and used in the detection methods of the invention. In another preferred embodiment, PrPBP binding domains are expressed as fusion proteins containing both mouse and human PrPBP coding sequences. Again, these fusion proteins are used in the detection methods described herein.

In one working example, a biological sample known or suspected to contain a prion agent is tested as follows. The sample is incubated with either a detectably labeled PrPBP (or a fragment or analog thereof) or PrPBP fusion protein present in a known concentration. Following incubation with the PrPBP, the biological sample is treated with a protease according to standard techniques (e.g., as described in Prusiner et al., Science 216:136-144, 1982), and the resulting complex isolated or identified, for example, by filter binding, immunoprecipitation, gel electrophoretic resolution, sedimentation, or filtration. The isolated complex is then analyzed and measured according to standard methods. Any appropriate label which may be directly or indirectly visualized may be utilized in these detection assays including, without limitation, any radioactive, fluorescent, chromogenic (e.g., alkaline phosphatase or horseradish peroxidase), or chemiluminescent label, or a hapten (for example, digoxigenin or biotin) which may be visualized using a labeled, hapten-specific antibody or other binding partner (e.g., avidin). If immunological-based assays are performed, polyclonal or monoclonal antibodies produced against PrPBP (as described above) or PrP (Bendheim et al., Nature 310:418-21, 1984) may be used in any standard immunoassay format (e.g., ELISA, Western blot, or RIA assay) to measure complex formation. Examples of such immunoassays are described, e.g., in

Ausubel et al., supra. Samples found to contain increased levels of labeled complex compared to appropriate control samples are taken as indicating the presence of a prion protein, and are thus indicative of a prion-related disease. Moreover, because cells containing normal levels of PrP^C may be used as controls, a labeled secondary antibody directed against PrP may also be used in the absence of protease treatment, and a signal above the PrP^C threshold detected as a measurement of PrP^{Sc} in the biological sample. In yet another alternative that does not require protease treatment, PrPBP may be allowed to complex with prion proteins in a biological sample, and PrP^{Sc} complexes detected specifically using a labeled secondary PrP^{Sc}-specific antibody reagent.

In another detection assay, PrP^{Sc} in a biological sample is captured on a solid support (e.g., nitrocellulose, Immobilon, agarose beads, cyanogen-activated agarose, or tissue culture wells), and binding by PrPBP (or a fragment or analog thereof) or PrPBP fusion protein is detected. In the alternative, a PrPBP or a PrPBP fusion protein is bound to a solid support, and binding of PrP^{Sc} is detected. In these assay formats, detection is accomplished by any standard method as described above, for example, detection of PrP^{Sc} in association with a solid support is accomplished directly or indirectly using PrPBP as described above. Alternatively, PrP^{Sc} in a captured PrP^{Sc}:PrPBP complex may be detected using antibodies directed against PrP^{Sc} (Bendheim et al., Nature 310:418-21, 1984). Again, any appropriate label may be utilized, for example, any radioactive or fluorescent marker may be used or, alternatively, any enzyme marker may be utilized, including (without limitation) alkaline phosphatase or horseradish peroxidase, and detection may be accomplished by addition of a chromogenic or fluorogenic substrate.

As noted above, a PrPBP which binds PrP^C, but not PrP^{Sc} can be used to remove PrP^C from a biological sample. The remaining PrP^{Sc} in the sample can be quantitated by binding to a reagent, e.g., an antibody which binds both PrP^{Sc} and PrP^C. Thus, even a PrPBP which binds PrP^C, but does not bind PrP^{Sc} can be used in an assay for PrP^{Sc}.

In another detection assay, a detectably-labeled PrP^{Sc} is incubated with PrPBP, followed by incubation with a biological sample suspected of containing PrP^{Sc}. The resulting complexes are then measured and quantified according to conventional methods as described above. In this assay format, a decrease in the presence of labeled PrP^{Sc} found in the PrPBP:PrP^{Sc} complex as compared to control samples is taken as an indication that PrP^{Sc} is present in the biological sample, since unlabeled PrP^{Sc} found in the sample competitively displaces the labeled PrP^{Sc} present in the complex. A similar approach may be utilized in combination with a protease treatment step following PrPBP-PrP complex formation. In this case, any form of labeled PrP may be utilized, and, again, a decrease in the level of labeled complex (as compared to a control in the absence of sample) is taken as an indication that PrP^{Sc} is present in the biological sample being assayed.

Prion Decontamination

The methods and compositions described herein are useful for the decontamination of biological samples that are known or suspected of being contaminated with a prion protein. In particular, because PrPBP is capable of binding to PrP^{Sc}, biological samples may be incubated with PrPBP, and the PrPBP complexes removed using standard methods, e.g., secondary sequestration or separation methodologies. Alternatively, PrPBPs may simply be incubated with biological samples to complex with, and thereby inhibit, the effects of PrP.

Treatment of Prion-Related Diseases and Other Neurodegenerative Diseases

The methods and compositions of the invention also provide a means for treating or preventing prion diseases in mammals, including humans. This treatment may be accomplished directly, e.g., by treating the animal with compounds (for example, antagonists or agonists) which disrupt, suppress, attenuate, or neutralize the biological events associated with PrPBP:PrP^{Sc} complex formation. Such antagonists may be isolated using the molecules of the invention and the functional assays

described above. For example, the invention provides a means for the identification of compounds (e.g., peptides, small molecule inhibitors, or mimetics) that are capable of antagonizing PrPBP:PrP^{Sc} complex interactions. Such antagonists include, without limitation, PrPBP, PrPBP fragments or analogs that bind and inactivate the biological activity of PrP^{Sc}, anti-PrPBP antibodies, fragments or analogs of PrP, PrP^{Sc}, or PrP^{C} that interact with PrPBP and block PrPBP:PrP complex formation and disrupt the associated biological activity resulting from such complex formation; or peptide or non-peptide molecules, including pharmacological agents, that interfere and block the biological activity associated with PrP^{Sc}, PrPBP, or PrPBP:PrP^{Sc} complex formation, and that are found, e.g., in a cell extract, mammalian serum, or growth medium in which mammalian cells have been cultured.

Antagonist molecules useful in the invention include: molecules selected for their ability to bind with high affinity to PrP^{Sc} and render it incapable of recruiting more copies of itself from PrP^{C}; molecules capable of binding with high affinity to PrP^{C} and block its conversion to PrP^{Sc}; molecules capable of blocking the generation of cytotoxic signals by preventing the interaction of PrP^{C} with PrP^{Sc} or complexes thereof; molecules capable of blocking the generation of soluble toxic mediators (e.g., reactive oxygen species and expression of tumor necrosis factor) by neighboring cells expressing PrP^{C}; or any combination of these aforementioned mechanisms.

The efficacy of a candidate antagonist, e.g., PrPBP, is dependent upon its ability to interact with a prion protein. Such an interaction can be readily assayed using any number of standard binding techniques and functional assays (e.g., those described herein). In one particular example, candidate antagonists may be tested in vitro for interaction with PrP^{Sc} and their ability to modulate PrP^{Sc}-mediated biological activity may be assayed by any of the functional tests described herein.

A molecule that promotes a decrease in PrPBP:PrP^{Sc} complex formation or a decrease in PrPBP:PrP^{Sc}-mediated biological activity in vitro is considered useful in the invention; such a molecule can be used, for example, as a therapeutic to treat or prevent the onset of a prion disease.

Evaluation of whether a test antagonist confers protection against the development of a prion disease in vivo generally involves using an animal known to develop such a disease (e.g., Chandler, Lancet 6:1378-1379, 1961; Eklund et al., J. Infectious Disease 117:15-22, 1967; Field, Brit. J. Exp. Path. 8:129-239, 1969). An appropriate animal (for example, a mouse or hamster) is treated with the test compound according to standard methods, and a reduced incidence or delayed onset of a prion-related illness, compared to untreated control animals, is detected as an indication of protection. The test compound may be administered to an animal which has previously been injected with a prion agent or, alternatively, the test compound may be tested for its ability to neutralize a prion agent by pre-incubating the prion and the compound and injecting the prion/compound mixture into the test animal. A molecule (e.g., an antagonist as described above) that is used to treat or prevent a prion disease is referred to as an "anti-prion therapeutic."

An anti-prion therapeutic according to the invention may be administered with a pharmaceutically-acceptable diluent, carrier, or excipient, in unit dosage form. For example, conventional pharmaceutical practice may be employed to provide suitable formulations or compositions to administer such anti-prion therapeutics to animals suffering from or presymptomatic for a prion disease, or at risk for developing a prion disease. Any appropriate route of administration can be employed, for example, parenteral, intravenous, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, intranasal, aerosol, or oral administration.

Methods well known in the art for making formulations are found in, for example, "Remington's Pharmaceutical Sciences." Formulations for parenteral administration can, for example, contain excipients, sterile water, or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated napthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers can be used to control the release of the compounds. Other potentially useful parenteral

delivery systems for anti-prion therapeutic compounds include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation can contain excipients, for example, lactose, or can be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or can be oily solutions for administration in the form of nasal drops, or as a gel.

The methods of the present invention may be used to reduce or prevent the disorders described herein in any animal, for example, humans, domestic pets, or livestock. Where a non-human animal is treated, the anti-prion therapeutic employed is preferably specific for that species.

Other Embodiments

In general, the invention includes any PrPBP which may be isolated as described herein using a PrP-AP fusion protein or which is readily isolated by homology screening or PCR amplification using the murine PrPBP described above. Also included in the invention are PrP-AP fusion proteins and PrPBPs which are modified in ways which do not abolish their binding activity (assayed, for example as described herein). Such changes may include certain mutations, deletions, insertions, or post-translational modifications, or may involve the inclusion of the protein (for example, the PrPBP) as one component of a larger fusion protein.

Thus, in other embodiments, the invention includes any protein which is substantially identical to a PrPBP polypeptide and which binds an appropriate PrP (assayed, for example, as described herein). Such homologs include other substantially pure naturally-occurring mammalian PrPBPs as well as allelic variants; natural mutants; induced mutants; proteins encoded by DNA that hybridizes to the murine PrPBP DNA sequence under high stringency conditions or, less preferably, under low stringency conditions (e.g., washing at 2X SSC at 40°C with a probe length of at least 40 nucleotides); and proteins specifically bound by antisera directed to a PrPBP. The term also includes chimeric polypeptides that include a PrPBP portion.

The invention further includes analogs of any naturally-occurring PrPBP polypeptide. Analogs can differ from the naturally-occurring PrPBP protein by amino acid sequence differences, by post-translational modifications, or by both. Again, a PrPBP analog according to the invention must retain the capability to bind a PrP. Analogs of the invention will generally exhibit at least 85%, more preferably 90%, and most preferably 95% or even 99% identity with all or part of a naturally-occurring PrPBP amino acid sequence. The length of sequence comparison is at least 15 amino acid residues, preferably at least 25 amino acid residues, and more preferably more than 35 amino acid residues. Modifications include in vivo and in vitro chemical derivatization of polypeptides, e.g., acetylation, carboxylation, phosphorylation, or glycosylation; such modifications may occur during polypeptide synthesis or processing or following treatment with isolated modifying enzymes. Analogs can also differ from the naturally-occurring PrPBP by alterations in primary sequence. These include genetic variants, both natural and induced (for example, resulting from random mutagenesis by irradiation or exposure to ethanemethylsulfate or by site-specific mutagenesis as described in Sambrook, Fritsch and Maniatis, Molecular Cloning: A Laboratory Manual (2d ed.), CSH Press, 1989, or Ausubel et al., supra). Also included are cyclized peptides, molecules, and analogs which contain residues other than L-amino acids, e.g., D-amino acids or non-naturally occurring or synthetic amino acids, e.g., β or γ amino acids.

In addition to full-length polypeptides, the invention also includes PrPBP fragments. As used herein, the term "fragment," means at least 3 contiguous amino acids, preferably, at least 5 contiguous amino acids, more preferably, at least 20 contiguous amino acids, even more preferably, at least 30 contiguous amino acids, yet more preferably, at least 50 contiguous amino acids, and, most preferably, at least 60 to 80 or more contiguous amino acids. Fragments of PrPBPs can be generated by methods known to those skilled in the art or may result from normal protein

processing (e.g., removal of amino acids from the nascent polypeptide that are not required for biological activity or removal of amino acids by alternative mRNA splicing or alternative protein processing events).

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each independent publication or patent application was specifically and individually indicated to be incorporated by reference.

Other embodiments are within the scope of the claims.

What is claimed is:

Claims

1. A method of identifying a prion protein binding protein (PrPBP), said method comprising

a) contacting a cell or biological sample with a detectable prion protein (PrP) fusion protein under conditions that allow complex formation between said fusion protein and a PrPBP; and

b) detecting said complex, thereby identifying said PrPBP.

2. The method of claim 1, wherein said detectable PrP fusion protein binds to said PrPBP with high affinity.

3. The method of claim 1, wherein said detectable PrP fusion protein exhibits conformation dependent binding to said PrPBP.

4. The method of claim 1, wherein said detectable PrP fusion protein comprises a prion protein fused to alkaline phosphatase.

5. The method of claim 1, wherein said PrPBP is from a human, a domesticated farm animal, or a pet species.

6. The method of claim 1, wherein said PrPBP is from a mammal.

7. The method of claim 6, wherein said mammal is a cow, sheep, mouse, or goat.

8. The method of claim 1, wherein said method further comprises determining whether said PrPBP binds in a competitive manner to a PrP or PrP fusion protein.

9. The method of claim 1, wherein said method further comprises determining the affinity with which said PrPBP binds to a PrP or PrP fusion protein.

10. The method of claim 1, wherein said method further comprises determining whether binding of a PrP or PrP fusion protein to said PrPBP inhibits cell proliferation or promotes cell death.

11. A method for identifying a nucleic acid molecule which encodes a PrPBP, said method comprising:

a) providing a population of cells expressing a pool of nucleic acid molecules, said cells not normally expressing PrP on their cell surfaces;

b) exposing said population of cells to detectably labeled PrP; and

c) identifying a cell which binds detectably labeled PrP, thereby identifying a cell comprising a nucleic acid molecule which encodes a PrPBP.

12. The method of claim 11, wherein said nucleic acid molecules are cDNA molecules.

13. The method of claim 11, wherein said nucleic acid molecules are mammalian nucleic acid molecules.

14. The method of claim 11, wherein said cells are frog oocytes.

15. A prion protein fusion protein comprising PrP covalently bound to a detectable protein.

16. The fusion protein of claim 15, said fusion protein exhibiting high affinity binding to a PrPBP.

17. The fusion protein of claim 15, said fusion protein exhibiting conformation dependent binding to a PrPBP.

18. The fusion protein of claim 15, wherein said detectable protein is alkaline phosphatase.

19. The fusion protein of claim 15, wherein said prion protein is from a human, a domesticated farm animal, or a pet species.

20. The fusion protein of claim 15, wherein said prion protein is from a mammal.

21. The fusion protein of claim 20, wherein said mammal is a cow, sheep, mouse, or goat.

22. The fusion protein of claim 15, wherein said fusion protein is soluble.

23. The fusion protein of claim 15, wherein said fusion protein is a secreted protein.

24. A method for detecting PrP^{Sc} in a biological sample, said method comprising:

a) contacting a biological sample with a PrPBP or PrP-binding fragment or analogue thereof; and

b) detecting complex formation between said PrPBP and PrP^{Sc} of said biological sample, said complex formation indicating the presence of PrP^{Sc} in said biological sample.

25. The method of claim 24, wherein said detecting step is carried out using a labeled PrP-specific antibody and a signal greater than that generated by a control sample lacking PrP^{Sc} is an indication of the presence of PrP^{Sc} in said biological sample.

26. The method of claim 24, wherein said detecting step is carried out using a labeled PrPBP and a signal greater than that generated by a control sample lacking PrP^{Sc} is an indication of the presence of PrP^{Sc} in said biological sample.

27. The method of claim 24, wherein said detecting step is carried out using a labeled PrP^{Sc}-specific antibody.

28. The method of claim 24, wherein said method further comprises destroying PrP^{C} in said biological sample following said contacting step and prior to said detecting step.

29. The method of claim 28, wherein said PrP^{C} is destroyed by proteolytic degradation.

30. The method of claim 24, wherein said PrPBP is detectable.

31. The method of claim 30, wherein said PrPBP is a detectable fusion protein.

32. The method of claim 31, wherein said PrPBP fusion protein is a PrPBP-alkaline phosphatase fusion protein.

33. The method of claim 30, wherein said PrPBP is labeled with a radioactive, fluorescent, chromogenic, or chemiluminescent label, or a hapten.

34. The method of claim 24, wherein said complex formation is detected using a labeled PrPBP-specific antibody.

35. The method of claim 24, wherein said complex formation is detected by filter binding, immunoprecipitation, gel electrophoretic resolution, sedimentation, or filtration.

36. The method of claim 24, wherein said PrP^{Sc} is immobilized on a solid support.

37. The method of claim 24, wherein said PrPBP or PrP-binding fragment or analogue thereof is immobilized on a solid support.

38. The method of claim 37, wherein said complex formation is detected using a PrP-specific antibody.

39. A method for detecting PrP^{Sc} in a biological sample, said method comprising:

a) contacting said biological sample with a PrPBP:labeled PrP complex;

b) destroying PrP^{C} in said biological sample; and

c) detecting displacement of labeled PrP in said PrPBP:PrP complex by unlabeled PrP^{Sc} present in said biological sample, said displacement indicating the presence of PrP^{Sc} in said biological sample.

40. The method of claim 39, wherein said labeled PrP is labeled PrP^{Sc}.

41. The method of claim 39, wherein said labeled PrP is a PrP fusion protein.

42. The method of claim 41, wherein said PrP fusion protein is a PrP-alkaline phosphatase fusion protein.

43. The method of claim 39, wherein said PrP^C is destroyed by proteolytic degradation.

44. The method of claim 39, wherein said complex or said PrP^{Sc} is immobilized on a solid support.

45. The method of claim 39, wherein said PrP is labeled with a radioactive, fluorescent, chromogenic, or chemiluminescent label, or a hapten.

46. The method of claim 39, wherein said complex is detected by filter binding, immunoprecipitation, gel electrophoretic resolution, sedimentation, or filtration.

47. A method for detecting PrP^{Sc} in a biological sample, said method comprising:

a) contacting said biological sample with a PrPBP:labeled PrP complex; and

b) detecting displacement of labeled PrP in said PrPBP:PrP complex by unlabeled PrP^{Sc} present in said biological sample, a decrease in said complex-associated label relative to a control sample lacking PrP^{Sc} being an indication of the presence of PrP^{Sc} in said biological sample.

48. The method of claim 24 or 39, wherein said PrPBP is from a human, a domesticated farm animal, or a pet species.

49. The method of claim 24 or 39, wherein said PrPBP is a mammalian PrPBP.

50. The method of claim 49, wherein said mammal is a cow, sheep, mouse, or goat.

51. The method of claim 24 or 39, wherein said biological sample is from a human, a domesticated farm animal, or a pet species.

52. The method of claim 24 or 39, wherein said PrPBP is soluble.

53. The method of claim 24 or 39, wherein said PrPBP is a cadherin.

54. The method of claim 53, wherein said cadherin is protocadherin-43.

55. The method of claim 53, wherein said cadherin is OB-cadherin-1.

56. The method of claim 54, wherein said cadherin has the sequence of SEQ ID NO: 2.

57. The method of claim 55, wherein said cadherin has the sequence of SEQ ID NO: 8 or 9.

58. A kit for detecting PrP^{Sc} in a biological sample, said kit comprising a PrPBP or a PrP-binding fragment or analogue thereof.

59. The kit of claim 58, said kit further comprising a PrP-specific antibody.

60. The kit of claim 59, wherein said PrP-specific antibody is a PrP^{Sc}-specific antibody.

61. The kit of claim 58, wherein said PrPBP is detectable.

62. The kit of claim 61, wherein said PrPBP is a detectable fusion protein.

63. The kit of claim 62, wherein said PrPBP is a PrPBP-alkaline phosphatase fusion protein.

64. The kit of claim 61, wherein PrPBP is labeled with a radioactive, fluorescent, chromogenic, or chemiluminescent label, or a hapten.

65. The kit of claim 58, wherein said PrPBP is immobilized on a solid support.

66. The kit of claim 58, wherein said kit further comprises a labeled PrP.

67. The kit of claim 58, wherein said PrPBP is from a human, a domesticated farm animal, or a pet species.

68. The kit of claim 58, wherein said PrPBP is a mammalian PrPBP.

69. The kit of claim 68, wherein said mammal is a cow, sheep, mouse, or goat.

70. The kit of claim 58, wherein said PrPBP is a cadherin.

71. The kit of claim 70, wherein said cadherin is protocadherin-43.

72. The kit of claim 70, wherein said cadherin is OB-cadherin-1.

73. The kit of claim 71, wherein said cadherin has the sequence of SEQ ID NO: 2.

74. The kit of claim 72, wherein said cadherin has the sequence of SEQ ID NO: 8 or 9.

75. A method for identifying a potential therapeutic compound for the treatment of a prion disease, said method comprising:

a) measuring the binding of a selected PrPBP to PrP^{Sc} or PrP^{C} in the presence of a test compound; and

b) measuring the binding of said selected PrPBP to PrP^{Sc} or PrP^{C} in the absence of said test compound;

wherein a level of binding of said selected PrPBP to PrP^{Sc} or PrP^{C} in the presence of said test compound that is less than the level of binding of said selected PrPBP to PrP^{Sc} or PrP^{C} in the absence of said test compound is an indication that said test compound is a potential therapeutic compound for the treatment of a prion disease.

76. The method of claim 75, wherein the selected PrPBP is a cadherin.

77. The method of claim 76, wherein said cadherin is protocadherin-43.

78. The method of claim 76, wherein said cadherin is OB-cadherin-1.

79. The method of claim 77, wherein said selected PrPBP has the sequence of SEQ ID NO: 2.

80. The method of claim 78, wherein said selected PrPBP has the sequence of SEQ ID NO: 8 or 9.

81. The method of claim 75, wherein said prion disease affects a human, a domesticated farm animal, or a pet species.

82. The method of claim 75, wherein said prion disease affects a human, cow, sheep, or goat.

83. A method for inhibiting PrP^{Sc} in a biological sample, comprising contacting the biological sample with a PrPBP under conditions which allow the formation of complexes between PrP^{Sc} and said PrPBP.

84. The method of claim 83, wherein said method further comprises recovering the PrP^{Sc}:PrPBP complex from the biological sample.

85. A method for treating a prion disease in a mammal, the method comprising administering a therapeutically-effective amount of all or a PrP-binding portion of a PrPBP to said mammal.

86. The method of claim 85, wherein said PrPBP is a cadherin.

87. The method of claim 86, wherein the cadherin is protocadherin-43.

88. The method of claim 86, wherein the cadherin is OB-cadherin-1.

89. The method of claim 87, wherein said PrPBP has the sequence of SEQ ID NO: 2.

90. The method of claim 88, wherein said PrPBP has the sequence of SEQ ID NO: 8 or 9.

91. A method for treating a disorder associated with an undesirable level of interaction between PrP^C and a PrPBP in a mammal, the method comprising administering a therapeutically-effective amount of all or a PrP-binding portion of a PrPBP to said mammal.

92. The method of claim 91, wherein said PrPBP is a cadherin.

93. The method of claim 91, wherein said disorder is cancer.

94. The method of claim 91, wherein said disorder is a neurodegenerative disorder.

95. The method of claim 91, wherein said disorder is an immunological disorder.

96. The method of claim 91, wherein said disorder comprises an abnormal proliferation or secretion of immunoglobulin.

97. The method of claim 91, wherein said disorder is a lymphoma, multiple myeloma, monoclonal gammopathy, B cell-related autoimmune disease, myasthenia gravis, or rheumatoid arthritis.

98. The method of claim 92, wherein said cadherin is protocadherin-43.

99. The method of claim 92, wherein said cadherin is OB-cadherin-1.

100. The method of claim 98, wherein said PrPBP has the sequence of SEQ ID NO: 2.

101. The method of claim 99, wherein said PrPBP has the sequence of SEQ ID NO: 8 or 9.

102. The method of claim 91, wherein said mammal is a human, a domesticated farm animal, or a pet species.

103. The method of claim 91, wherein said mammal is a human, cow, sheep, or goat.

104. A method for detecting B lymphocytes in a biological sample, said method comprising:

a) contacting a biological sample with a PrPBP or PrP-binding fragment or analogue thereof; and

b) detecting complex formation between said PrPBP and said biological sample, said complex formation indicating the presence of B lymphocytes in said biological sample.

105. The method of claim 104, wherein said biological sample is a blood sample.

106. The method of claim 104, wherein said PrPBP is a cadherin.

107. The method of claim 104, wherein said cadherin is protocadherin-43.

108. The method of claim 107, wherein said PrPBP has the sequence of SEQ ID NO: 2.

109. A kit for detecting B lymphocytes, said kit comprising a PrPBP.

110. The kit of claim 109, wherein said PrPBP is a cadherin.

111. The kit of claim 110, wherein said cadherin is protocadherin-43.

112. The kit of claim 111, wherein said PrPBP has the sequence of SEQ ID NO: 2.

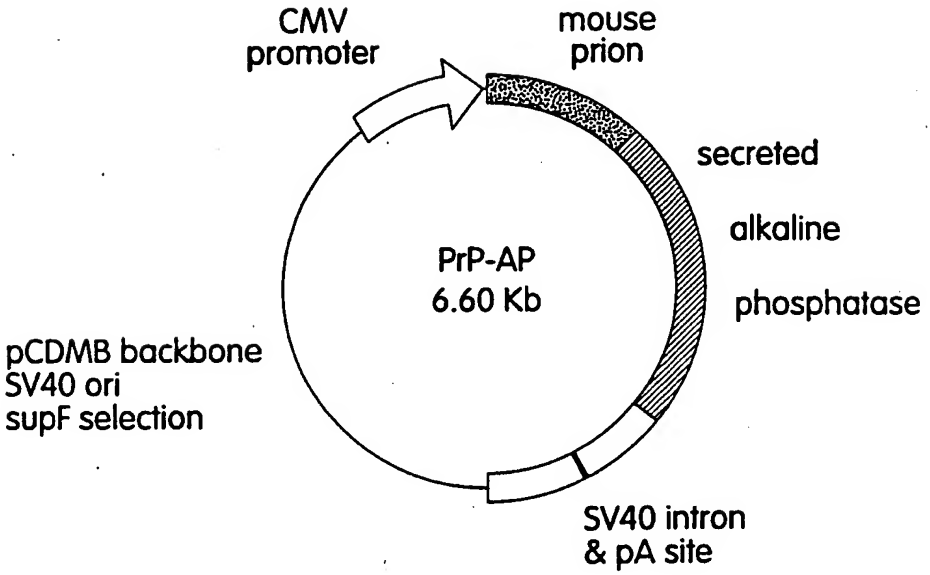
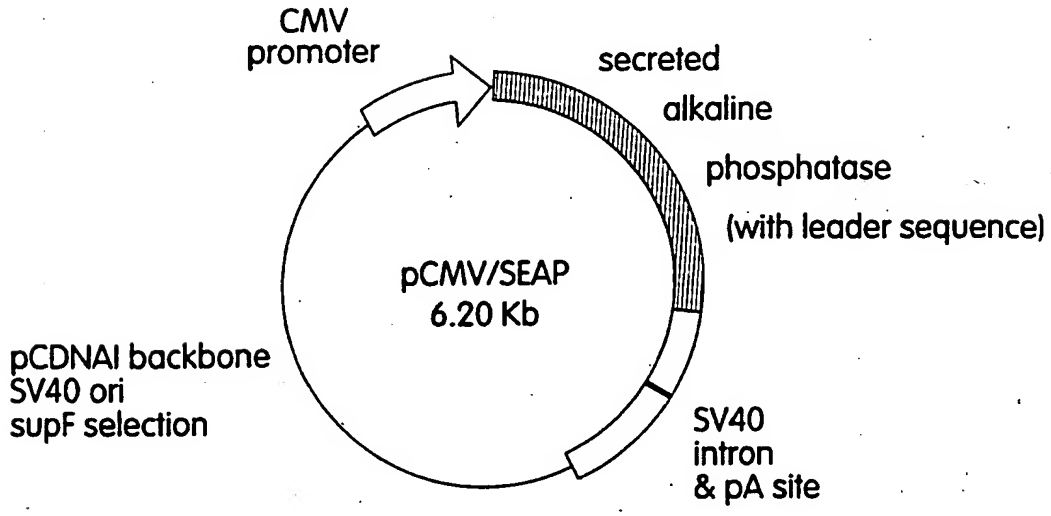

CMV
promoter
mouse
prion
secreted
alkaline
phosphatase
PrP-AP
6.60 Kb
pCDMB backbone
SV40 ori
supF selection
SV40 intron
& pA site

Fig. 1A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promoter
secreted
alkaline
phosphatase
(with leader sequence)
pCMV/SEAP
6.20 Kb
pCDNAI backbone
SV40 ori
supF selection
SV40
intron
& pA site

Fig. 1B

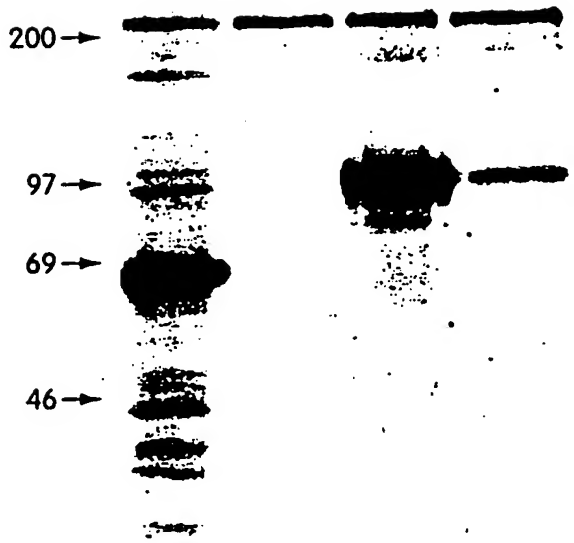


Fig. 2

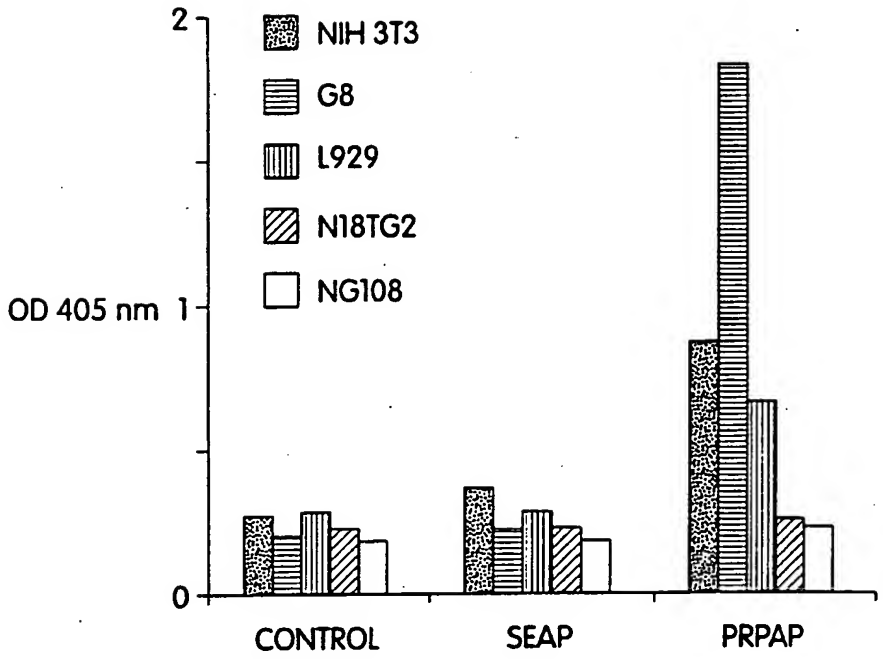


Fig. 3

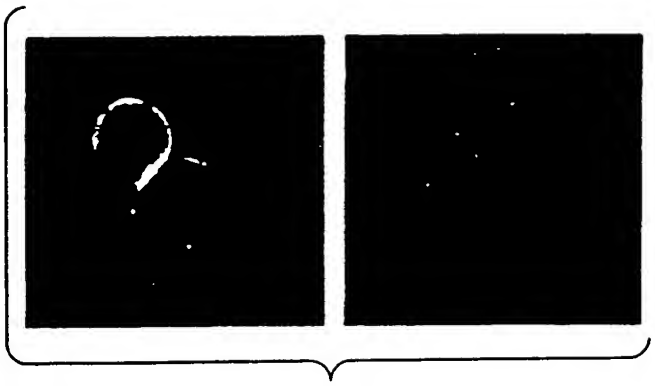


Fig. 4

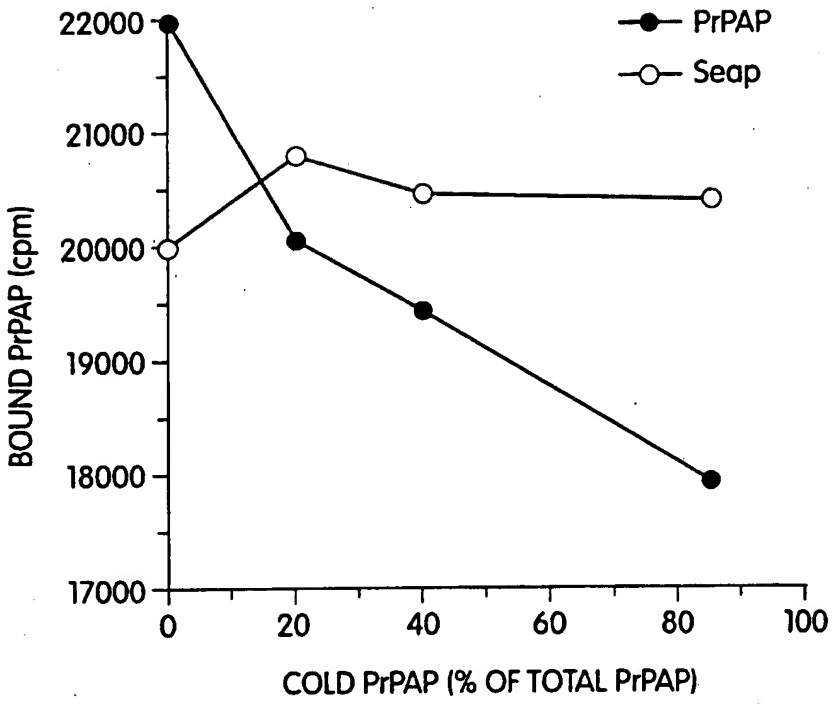

PrPAP
Seap
22000
21000
20000
19000
18000
17000
BOUND PrPAP (cpm)
0
20
40
60
80
100
COLD PrPAP (% OF TOTAL PrPAP)

Fig. 5

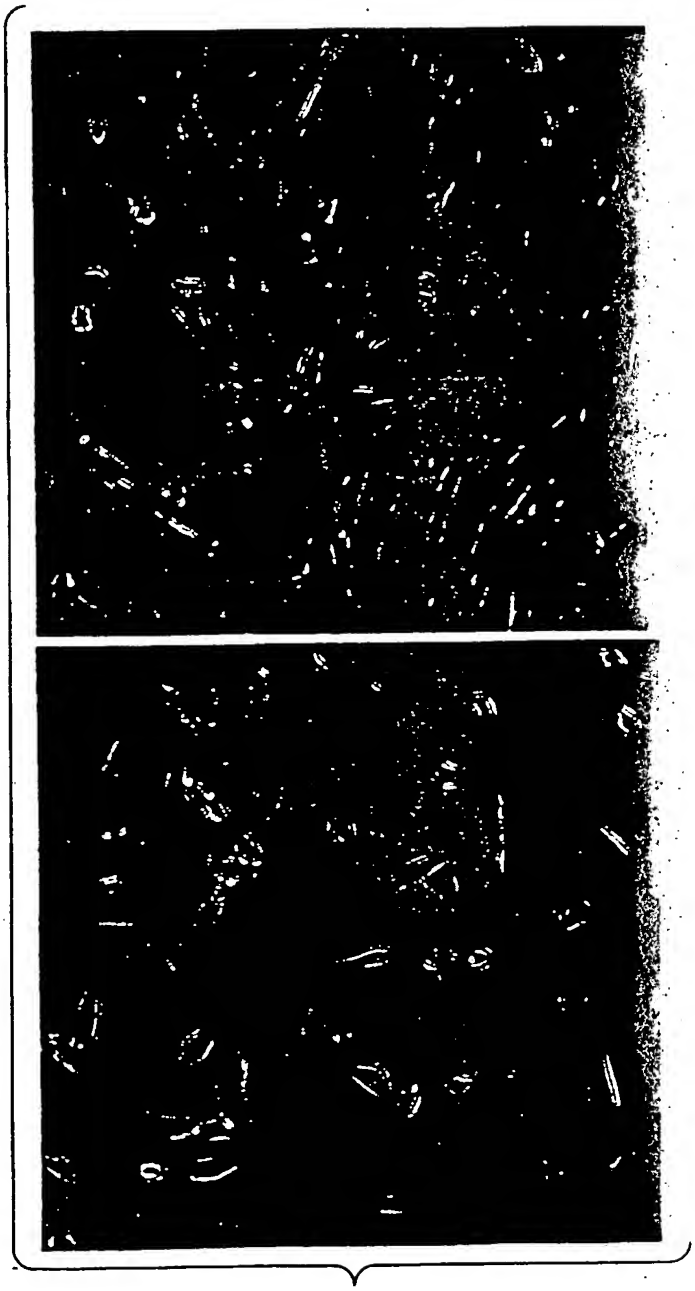
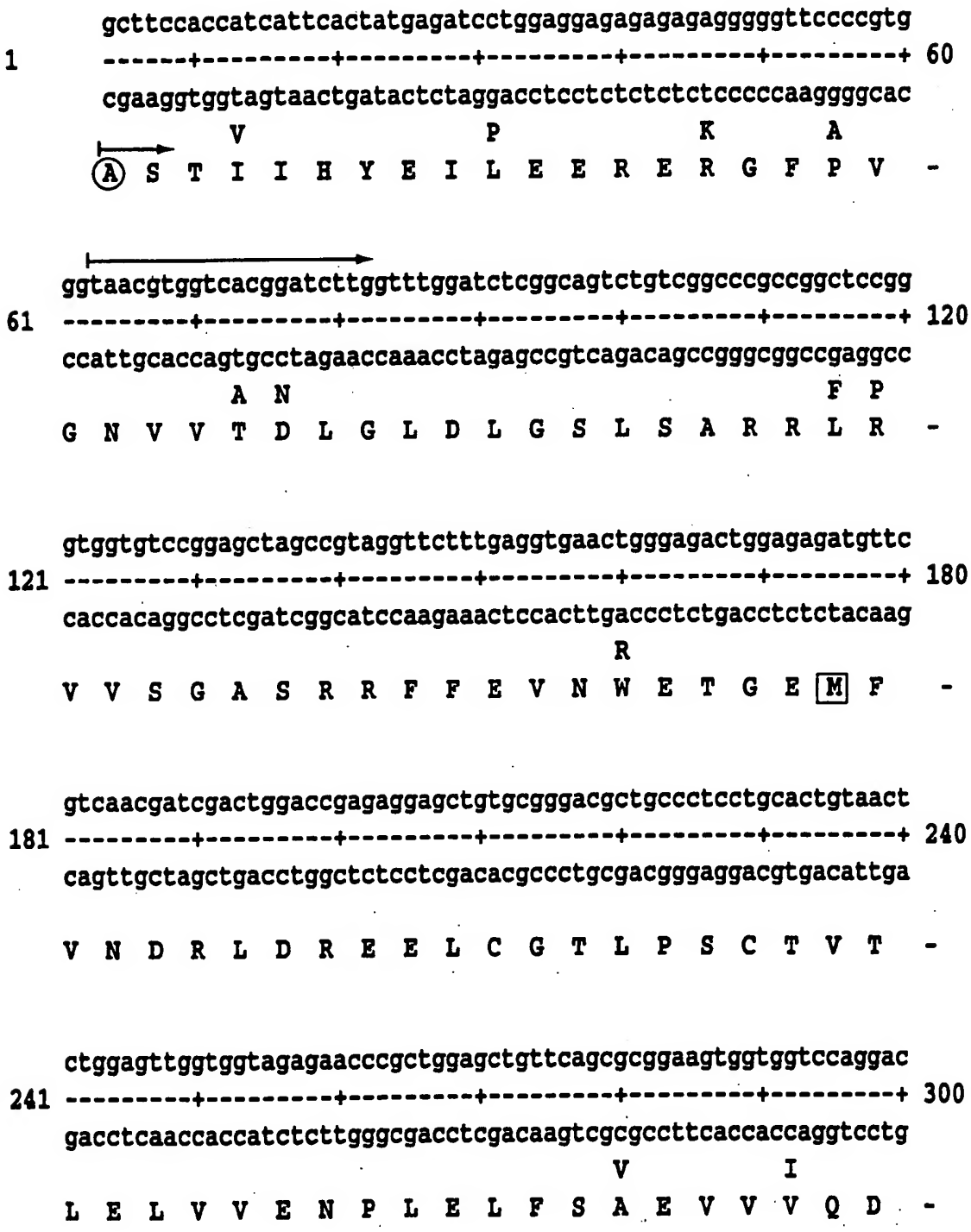


Fig. 6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1 ------+---------+---------+---------+---------+---------+ 60
cgaaggtggtagtaactgatactctaggacctcctctctctctcccccaaggggcac
V P K A
A S T I I H Y E I L E E R E R G F P V -
ggtaacgtggtcacggatcttggtttggatctcggcagtctgtcggcccgccggctccgg
61 ---------+---------+---------+---------+---------+---------+ 120
ccattgcaccagtgcctagaaccaaacctagagccgtcagacagccgggcggccgaggcc
A N F P
G N V V T D L G L D L G S L S A R R L R -
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121 ---------+---------+---------+---------+---------+---------+ 180
caccacaggcctcgatcggcatccaagaaactccacttgaccctctgacctctctacaag
R
V V S G A S R R F F E V N W E T G E M F -
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181 ---------+---------+---------+---------+---------+---------+ 240
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V N D R L D R E E L C G T L P S C T V T -
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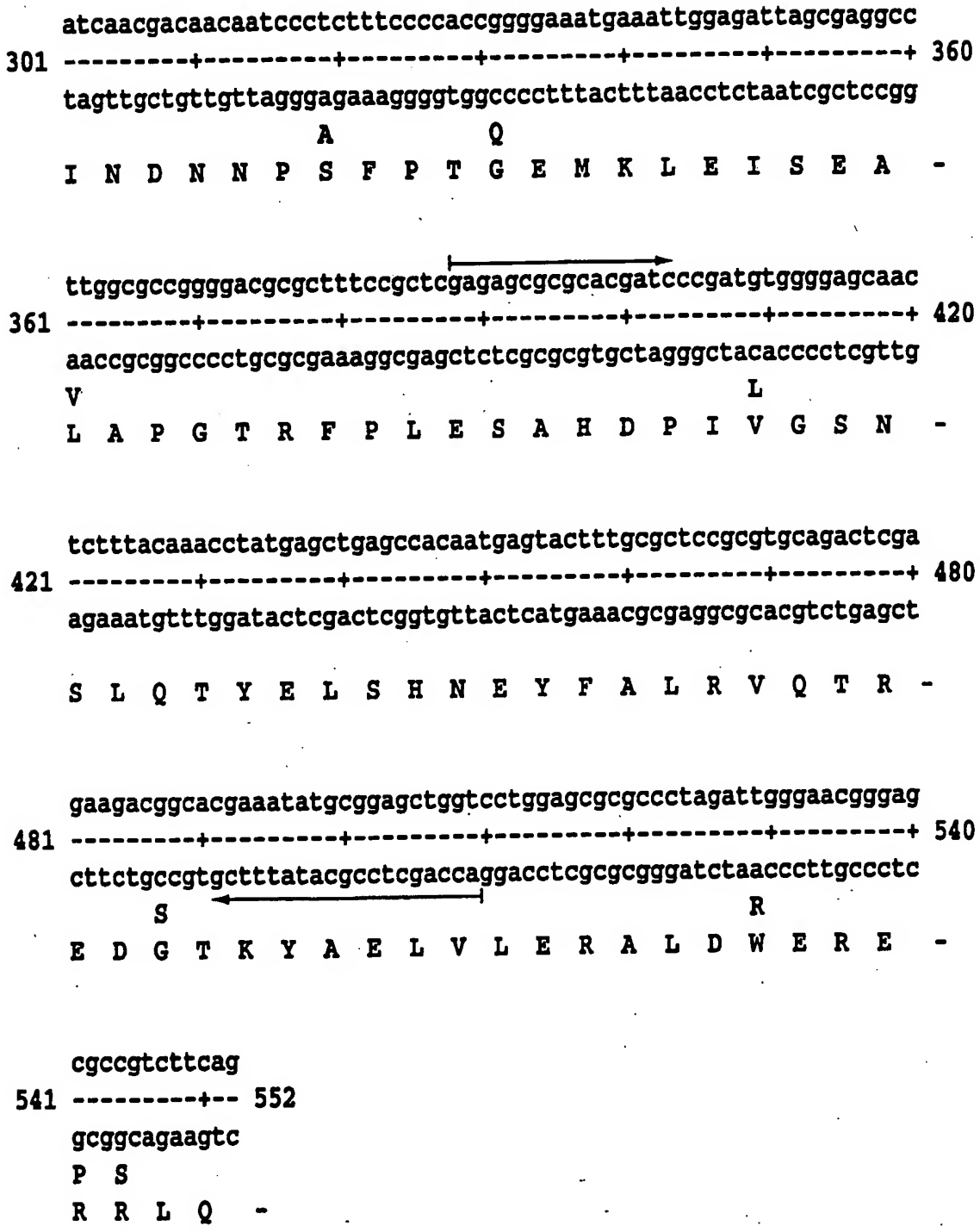
241 ---------+---------+---------+---------+---------+---------+ 300
gacctcaaccaccatctcttgggcgacctcgacaagtcgcgccttcaccaccaggtcctg
V I
L E L V V E N P L E L F S A E V V V Q D -

Fig. 7A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301 ---------+---------+---------+---------+---------+---------+ 360
tagttgctgttgttagggagaaaggggtggccccttttactttaacctctaatcgctccgg
A Q
I N D N N P S F P T G E M K L E I S E A -
ttggcgccggggacgcgctttccgctcgagagcgcgcacgatcccgatgtggggagcaac
361 ---------+---------+---------+---------+---------+---------+ 420
aaccgcggcccctgcgcgaaaggcgagctctcgcgcgtgctagggctacacccctcgttg
V L
L A P G T R F P L E S A H D P I V G S N -
tctttacaaacctatgagctgagccacaatgagtactttgcgctccgcgtgcagactcga
421 ---------+---------+---------+---------+---------+---------+ 480
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S L Q T Y E L S H N E Y F A L R V Q T R -
gaagacggcacgaaatatgcggagctggtcctggagcgcgccctagattgggaacgggag
481 ---------+---------+---------+---------+---------+---------+ 540
cttctgccgtgctttatacgcctcgaccaggacctcgcgcgggatctaacccttgccctc
S R
E D G T K Y A E L V L E R A L D W E R E -
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541 ---------+-- 552
gcggcagaagtc
P S
R R L Q -

Fig. 7B

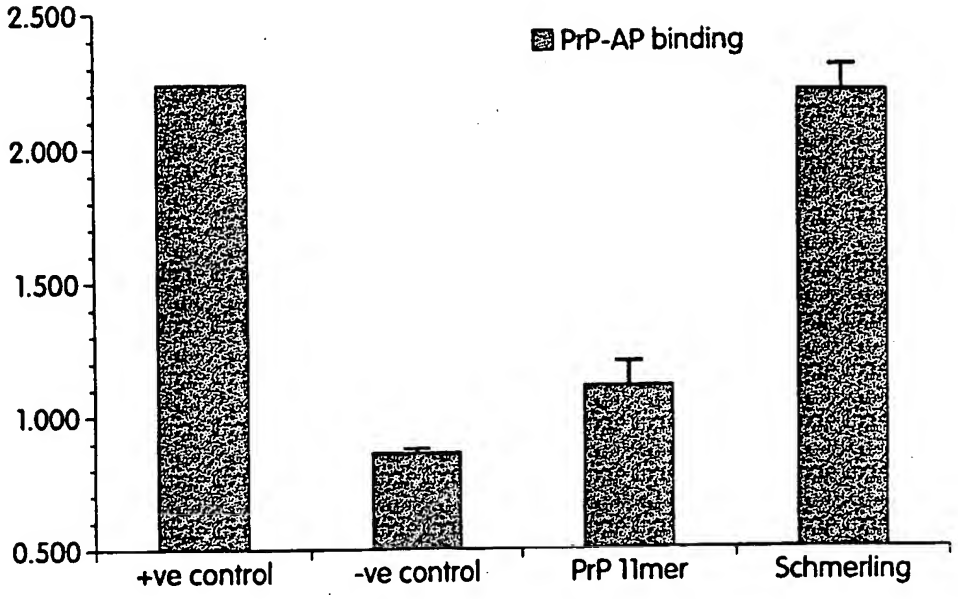
2.500
2.000
1.500
1.000
0.500
PrP-AP binding
+ve control
-ve control
PrP 11mer
Schmerling

Fig. 8A

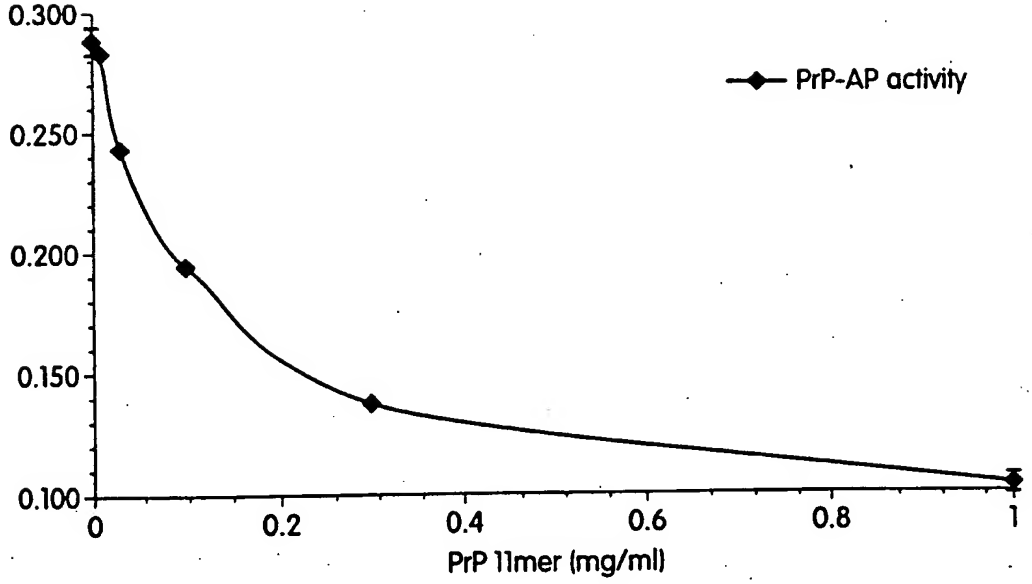
0.300
0.250
0.200
0.150
0.100
PrP-AP activity
0
0.2
0.4
0.6
0.8
1
PrP 11mer (mg/ml)

Fig. 8B

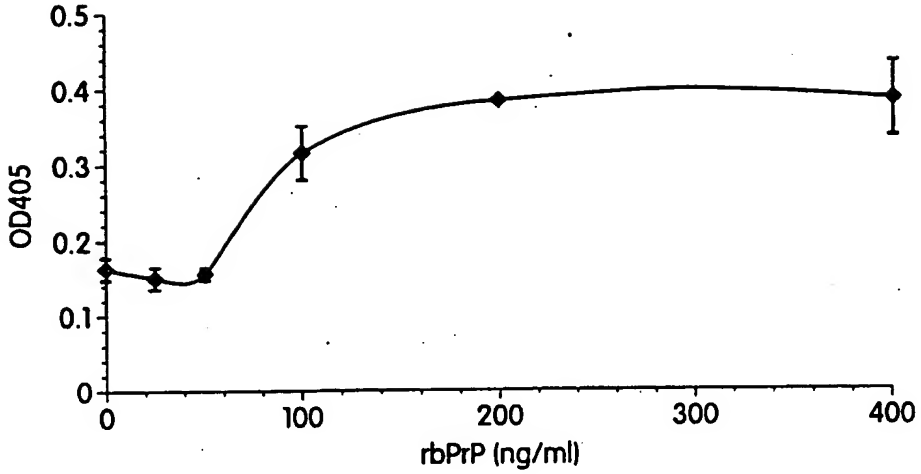
0.5
0.4
0.3
0.2
0.1
0
OD405
0
100
200
300
400
rbPrP (ng/ml)

Fig. 9

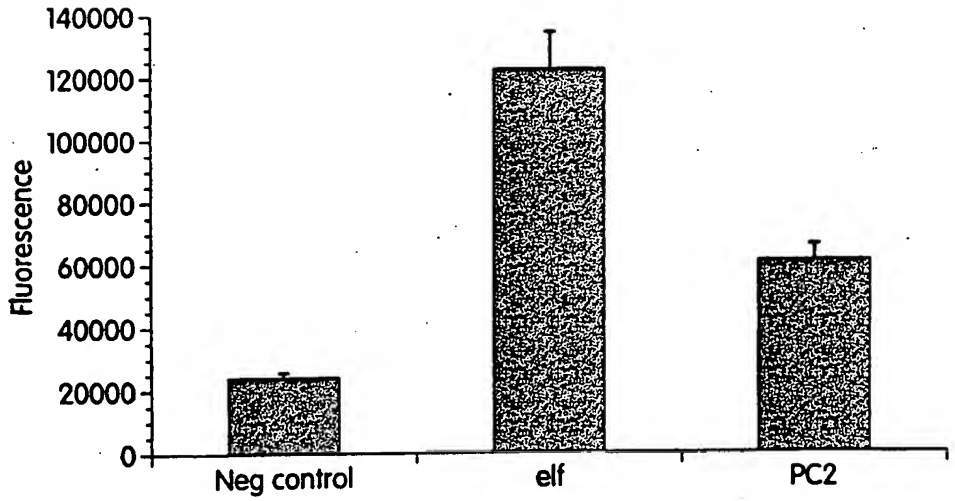
140000
120000
100000
80000
60000
40000
20000
0
Fluorescence
Neg control
elf
PC2

Fig. 10

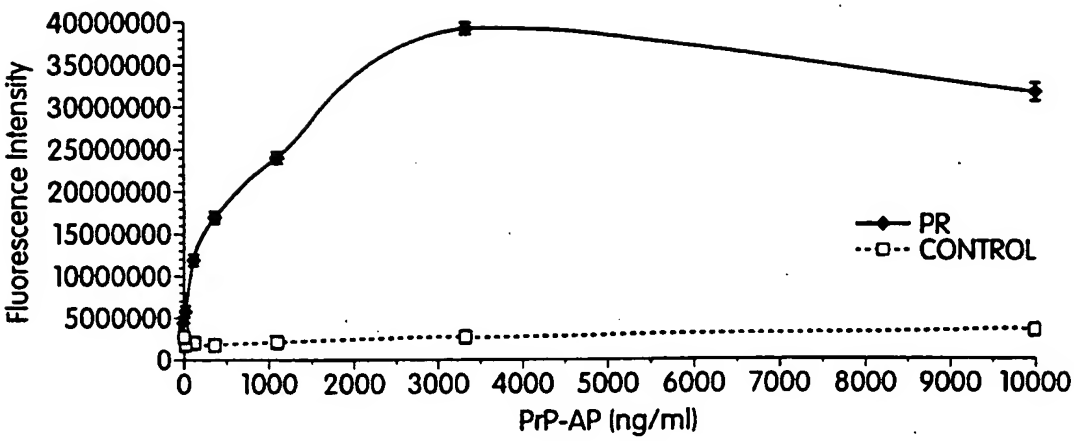
Fluorescence Intensity
40000000
35000000
30000000
25000000
20000000
15000000
10000000
5000000
0
0 1000 2000 3000 4000 5000 6000 7000 8000 9000 10000
PrP-AP (ng/ml)
PR
CONTROL

Fig. 11

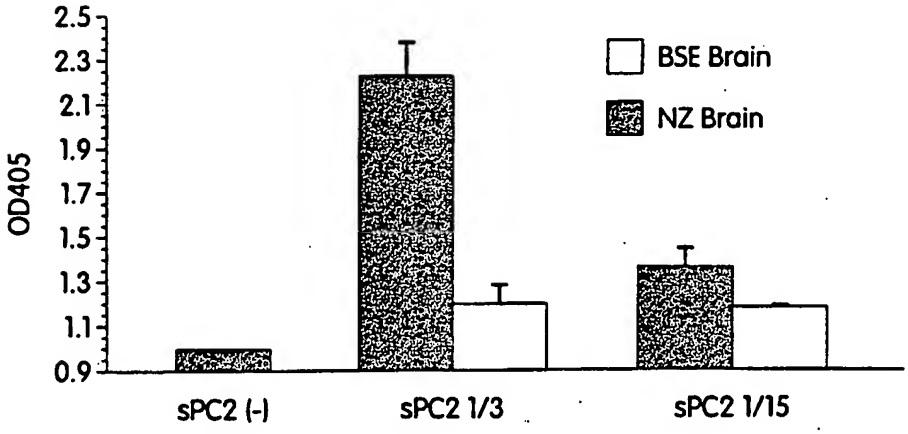
OD405
2.5
2.3
2.1
1.9
1.7
1.5
1.3
1.1
0.9
BSE Brain
NZ Brain
sPC2 (-)
sPC2 1/3
sPC2 1/15

Fig. 12

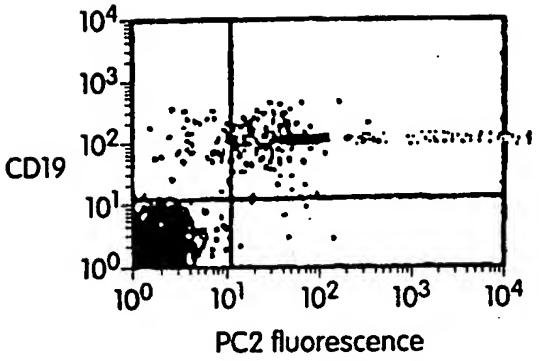

Fig. 13A

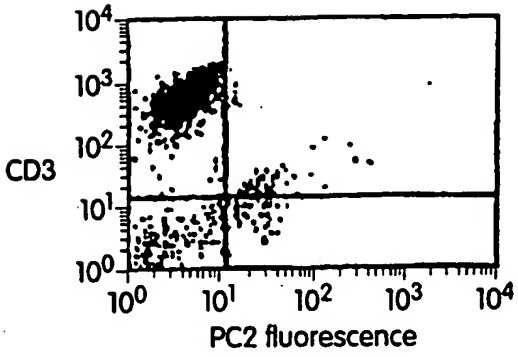

Fig. 13B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/17927

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/134.1, 185.1, 192.1; 436/501, 513, 518; 530/387.1, 389.1; 536/23.4

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS, CABA, CAPLUS, EMBASE, LIFESCI, MEDLINE, SCISEARCH, USPATFULL, JAPIO

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,679,530 A (BRENTANI et al.) 21 October1997, see entire reference.	1-10, 15-74
Y	US 5,750,361 A (PRUSINER et al.) 12 May1998, see entire reference.	1-10, 15-74
Y	US 4,806,627 A (WISNIEWSKI et al.) 21 February1989, see entire reference.	1-10, 15-74
X	WO 97/45746 A2 (MCGILL UNIVERSITY) 04 December 1997, see entire reference.	1-10, 15-74

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 27 SEPTEMBER 2000	Date of mailing of the international search report 26 OCT 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Cynthia Lawrence Lee RODNEY P. SWARTZ, PH.D. Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/17927

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-10, 15-74

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/17927

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

A61K 39/00, 39/40, 39/395; G01N 33/543, 33/563, 33/566; C07K 16/00; C07H 21/04

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/134.1, 185.1, 192.1; 436/501, 513, 518; 530/387.1, 389.1; 536/23.4

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-10, 15-74, drawn to prion protein binding protein, method of identifying, and first method of use.
Group II, claims 11-14, drawn to method of identifying a nucleic acid molecule.
Group III, claims 75-82, drawn to method of identifying inhibitors.
Group IV, claims 83-103, drawn to method of treating mammals.
Group V, claims 104-112, drawn to method of detecting B-lymphocytes.

The inventions listed as Groups I-V do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The prion protein binding protein (PPBP) is a single inventive concept, and as such, a method of making and a first method of use are included in the first invention. Group II is drawn to a structurally and functionally distinct molecule, DNA. Group III is claiming a second method of use. Group IV is claiming a third method of use. Group V is claiming a fourth method of use.